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HETEROCYCLISCHE 12-π- UND 14-π-SYSTEME, 27. MITTEILUNG. UNTERSUCHUNGEN ZUR SYNTHESE UND ZUM REAKTIONSVERHALTEN NEUER THIA-PSEUDO-PHENALENON-DERIVATE

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Zusammenfassung

Die Reaktion von 2-Methyl-benzo(b)thiophen *(1)* mit α-Chlor-dimethylether ergibt 2-Methyl-3-chloromethylbenzo(b)thiophen *(2)*, das nach bekannten Methoden in die Säure *(3)*, durch Cyclisierung und anschliessender Oxidation in *(4)* bzw. *(5)* überführt werden konnte. Die Alkylierung von *(5)* mit «Meerweinsalz» ergibt das Thia-pseudo-phenaleniumtetrafluoroborat *(6)* und in Anwesenheit einer Base unter Rearomatisierung *(6a)*. *(5)* bildet mit N-Nukleophilen die Derivate *(7a-7c, 8)*, mit C-Nukleophilen die Thiapseudophenafulvalene *(9, 10a-10d)*; darüberhinaus ist uns gelungen, die Thiapentapseudophenafulvalene *(11)* sowie *(12a-12d)* herzustellen, die durch spektroskopische Methoden identifiziert wurden.

Schlüsselwörter: *Heterocyclische 12-π-Elektronensysteme; heterocyclische 14-π-Elektronensysteme; 2-Methyl-benzo(b)thiophen; 2-Methyl-5-oxo-5H-naphtho (1.8-bc) thiophen; 2-Methyl-5-ethoxy-naphtho(1.8-bc) thiolium-kation; Reaktion der Thia-pseudo-phenalenone mit N- und C-Nukleophilen; Thia-penta-pseudophenafulvalene; 5-Ethoxy-2-methylennaphtho (1.8-bc) thiophen.*

Einführung

In Fortführung unserer Untersuchungen über Heteropseudophenalenone, ihrer Kationochemie, ihrer Fulvene und ihrer Hetero-penta-pseudophenafulvalene beschäftigten wir uns auch mit jenen 12-π-Molekülsystemen, welche im Tricyclus Schwefel als Hetero-atom[2-6] anstelle einer Doppelbindung, in α- Stellung jedoch eine CH_3 -Gruppe und in Position 5 die Garbonylgruppe enthielten. Die Reaktion der 12-π- elektronischen Thia-pseudophenalenone mit N-Nukleophilen, C-Nukleophilen, sowie die Synthese der Thia-penta-pseudophenafulvalene führten zu den vorgesehenen Derivaten. Es konnte gezeigt werden, dass die Thia-pseudophenalenone im elektronischen

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Grundzustand in bedeutendem Maße die zwitterionische dipolare Grenzstruktur einnehmen.

Ausgehend von 2-Methyl-benzo(b)thiophen *(1)*[6] wird mit einer aequimolaren Menge α -Chlor-dimethylether in Eisessig 2-Methyl-3-chlormethylbenzo(b)thiophen *(3)*[7] erhalten, welches nach bekannten Methoden in die 3-(2-Methyl-3-benzo(b)thienyl) propionsäure *(3)* überführt werden konnte; nach Cyclisierung zu *(4)* und dessen Dehydrierung[8,9,10] konnte 2-Methyl-thiapseudophenalenon-5 *(5)* isoliert werden. Das IR-Spektrum von *(5)* zeigte eine niederfrequente Carbonylvalenzschwingung bei 1635 cm^{-1}. Die niederfrequente Carbonylbande und die starke Nukleophilie weisen auf eine gegenüber einfachen α, β-ungesättigten Ketonen erhöhte Beteiligung der zwitterionischen, dipolaren Grenzstruktur am elektronischen Grundzustand der Carbonylgruppe hin[1,6,9,10]. Es war daher auch leicht möglich, das Keton *(5)* mit Hilfe von «Meerweinsalz» –dem Triethyloxoniumtetrafluoroborat – an der Carbonylgruppe zum 2-Methyl-5-ethoxy-thiapseudophenaleniumtetrafluoroborat *(6)* zu alkylieren; letzteres konnte allerdings aufgrund seiner Instabilität nicht isoliert werden und mit Hilfe der «Hünig-Base» Ethyldiisopropylamin wurde unter Eliminierung eines Protons bei gleichzeitiger Rearomatisierung des naphthalinoiden Anteils des Tricyclus *(6a)* erhalten. *(5)* reagierte mit m-

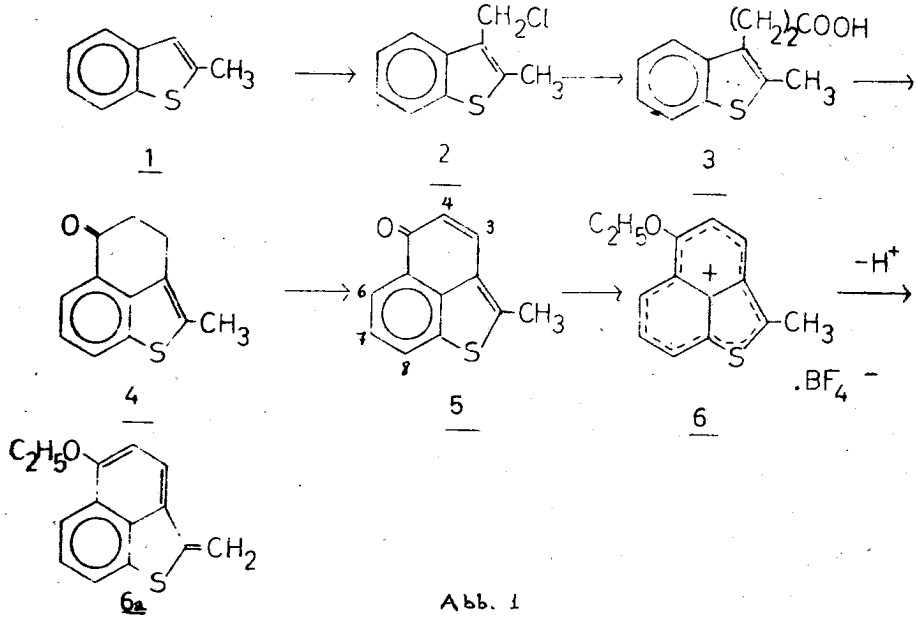
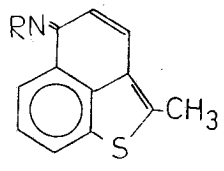


Abb. 1

Chlorphenylisocyanat in Gegenwart von $AlCl_3$ unter Abspatung von CO_2 zum Imino-derivat *(7a)*, mit den entsprechenden N-Nucleophilen wurden aus *(5)* unter saurer Katalyse die Imino-thiapseudophenalenone *(7b)*, *(7c)* und *(8)* erhalten. Die entsprechenden Thia-pseudophenafulvene *(10a - 10b)* waren nach zwei unterschiedlichen Methoden zugänglich: *(10a)* sowie *(10b)* wurden durch Kondensation von *(5)* mit Malonsäuredinitril bzw. mit Cyanessigsäuremethylester, *(10c)* und *(10d)* nach der Ketenmethode[9] aus *(5)* und den entsprechenden Ketenen unter Eliminierung von CO_2 erhalten. Im Falle von *(10d)* liegt aufgrund ^{1}H-NMR-spektroskopischer Untersuchungen ein Isomerengemisch vor, wobei allerdings jenes Isomere mit $R^1 = C(CH_3)_3$ als sehr voluminöser Gruppe und dadurch bedingter sterischer Hinderung überwiegen dürfte. Beim Isomeren mit $R^1 = C(CH_3)_3$ ist H^6 durch die Cyanogruppe nach tieferem Feld verschoben; H^4 zeigt sich dagegen bei relativ hohem Feld. Beim anderen Isomeren liegt H^6 im Aromatenbereich; H^4 ist stark entschirmt, aufgrund der benachbarten Cyanogruppe.



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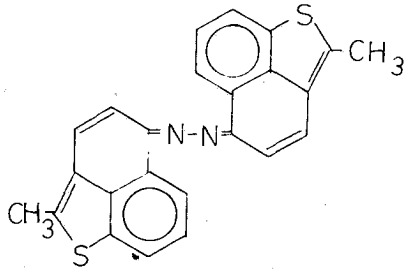
7a : R = m-Cl-C_6H_4
7b : R = C_6H_5
7c : R = p-CH_3-C_6H_4-SO_2NH



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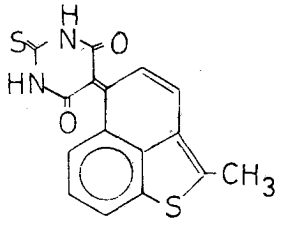

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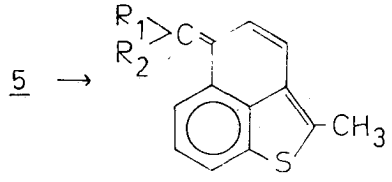

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10a : $R_1 = R_2 = CN$
10b : $R_1 = COOCH_3$ $R_2 = CN$
10c : $R_1 = R_2 = C_6H_5$
10d : $R_1 = C(CH_3)_3$ $R_2 = CN$
$R_1 = CN$ $R_2 = C(CH_3)_3$

Abb. 2

Als Vertreter der Thia-penta-pseudophenafulvalene wurden die Fulvalene *(11)* sowie *(12a - 12d)* synthetisiert und instrumental- analytisch untersucht; das intensiv dunkelblau kristalline *(11)* wurde aus *(5)* und Fluorenketen, die Fulvalene *(12a)* und *(12b)* nach der Acetanhydridmethode, *(12c)* durch Erhitzen der Reaktanten in Acetonitril als Lösungsmittel und *(12d)* durch Reaktion von *(5)* mit 2,3,4,5-Tetraphenyl-1-diazo-cyclopentadien in Gegenwart katalytischer Mengen $AlCl_3$ und in Xylol/Nitrobenzol als Lösungsmittel erhalten. Zusammenfassend kann gesagt werden, daß sowohl die Thia-pseudophenafulvene als auch die Thia-penta-pseudophenafulvalene sich als sehr stabile Verbindungen erwiesen haben.

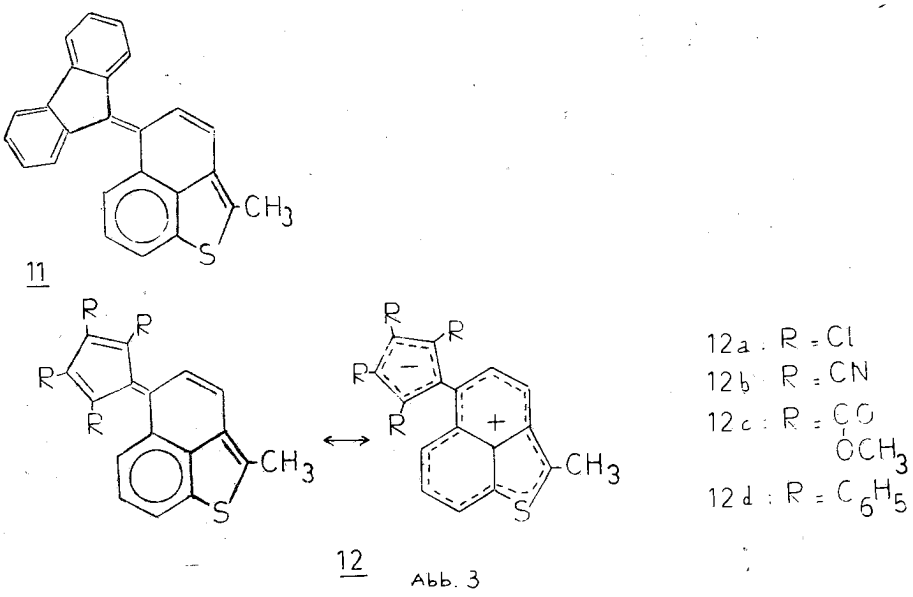

Abb. 3

Experimenteller Teil

Schmelzpunkte: nicht korrigiert, Schmelzpunktmikroskop der Fa. Reichert, Wien. - Massenspektren: Varian MAT 311, dessen inverse Nier-Johnson-Geometrie die Aufnahme der DADI-Spektren ermöglichte. ^{1}H-NMR-Spektren: Geräte Bruker-HX90 E und teilweise auch T-60-A; TMS als interner Standard. IR-Spektren: Gerät Perkin-Elmer 177. UV-Spektren: Gerät DMR 10 der Fa. Zeiss, Oberkochen. Die Elementaranalysen wurden mit dem C, H, N-Analysator der Fa. Heraeus, Hanau, ausgeführt. Säulenchromatographische Trennungen und Reinigungen wurden auf einer mit Kieselgel 60 «Merck» (0.063-0.20 mm) beschickten Säule von 80 cm Länge und einem Innendurchmesser von 2.5 cm durch-geführt, präparative schichtchromatographische Untersuchungen auf mit Kieselgel 60 GF_{254} «Merck» bestrichenen Glasplatten

(20×20cm). Die Lösungsmittel und Reagenzien wurden nach den üblichen Methoden getrocknet und gereinigt.

2-Methyl-3-chlormethyl-benzo(b)thiophen (2)

Zu einer Lösung von 14.8 g (0.1 mol) 2-Methyl-benzo(b)-thiophen *(1)* in 100 ml Eisessig werden 10 g (0.125 mol) Chloromethyl-methyl-ether in 10 ml Eisessig gegeben und 24 h stehengelassen; das auskristallisierte Rohprodukt wird aus Pentan umkristallisiert, weiße Kristalle. Schmp. 70°C; Ausb. 16.79 g (85%).

$C_{10}H_9ClS$ (196.70) Ber. C 61.06 H 5.13 Gef. C 60.92 H 5.04.

3(2-Methyl-benzo(b)thienyl)propionsäure (3)

16.79 g (0.085 mol) *(2)* reagieren mit 13.6 g (0.085 mol) Malonsäurediethylester in 15 ml wasserfreiem Ethanol zu 2-Methyl-3-(dicarbethoxy)-ethyl-benzo(b)thiophen, das anschließend mit 35 ml 1 N NaOH bei dreistündigem Erhitzen unter Rückfluß verseift worden ist. Der abgesaugte und getrocknete Niederschlag wird 20 min auf 200°C erhitzt, wobei unter Entwicklung von CO_2 die Säure entsteht; weiße Kristalle mit Schmp. 102 - 103°C (aus Petrolether 60-80°C); Ausb. 18.7 g (98%).

$C_{12}H_{12}O_2S$ (220.29) Ber. C 65.42 H 5.49 Gef. C 65.87 H 5.31.

2-Methyl-4.5-dihydro-5-oxo-5H-naphtho(1.8-bc) thiophen (4)

Eine Lösung von 1.60 g (7.25 mmol) *(3)* in 20 ml 1.2-Dichlorethan werden mit 5.0 ml Thionychlorid 30 min lang unter Rückfluß erhitzt; nach Abdestillieren des überschüssigen Thionylchlorids und des Lösungsmittels wird das Säurechlorid in 20 ml 1.2-Dichlorethan gelöst und bei 0°C 20 min lang mit 1.3 g Aluminiumchlorid behandelt. Anschließend wird die Lösung 30 min lang bei 0°C gerührt, sodann mit 50 ml conc. Salzsäure/Eis (1:1) hydrolysiert. Die organische Phase wird mit 15 proz. $NaHCO_3$-Lösung, danach mit Wasser gewaschen, das Lösungsmittel entfernt und der Rückstand aus 1.2-Dichlorethan/n-Hexan umkristallisiert. Gelbe Kristalle, Schmp. 88 - 89°C; Ausb. 0.44 g (30%).

$C_{12}H_{10}OS$ (202.28) Ber. C 71.26 H 4.98 Gef. C 70.87 H 5.05.

2-Methyl-5-oxo-5H-naphtho(1.8-bc)thiophen (5)

1. Methode: 2.0 g (10 mmol) *(4)* werden mit 15.35 g (62 mmol) Chloranil in 200 ml t-Butanol 3 h unter Rückfluß erhitzt, der Rückstand in Chloroform gelöst und mit 15 proz. Natriumhydrogenkarbonatlösung fünfmal gewaschen, anschließend mit Wasser und sodann aus Chloroform/n-Hexan umkristallisiert; gelbe Kristalle. Schmp. 76-77°C; Ausb. 1.0 g (50%).

2. Methode: 0.45 g (2.25 mmol) *(4)* und 0.76 g (4.5 mmol) Triphenylmethylperchlorat in wasserfreiem Eisessig werden 30 min lang bei 30°C gerührt und dann in 15 ml Wasser gegossen. Der organische Teil wird aus 30 ml Benzol extrahiert und die vereinigten benzolischen Auszüge mit 10 ml konz. Salzsäure extrahiert. Die Salzsäure-Phase wird auf 50 ml verdünnt

und das ausgefallene Produkt aus 1.1-Dichlormethan/n-Hexan umkristallisiert; gelbe Kristalle. Schmp. 76-77°C; Ausb. 0.34 g (75%).

IR (KBr): 1635 cm^{-1} (C=O). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.22 (d, H^3, I = 10 Hz), 7.05 (d, H^4, I = 10 Hz), 7.74 (dd, H^6, I = 8 Hz), 7.12 (t, H^7, I = 8 Hz), 7.38 (d, H^8, I = 8 Hz), 2.95 (s, α- CH_3). MS (70 eV): m/e (%) = 200 (90, M^+), 185 (25), 172 (23), 171 (100), 157 (41), 145 (10), 114 (18), 113 (20), 100 (16), 87 (8), 63 (9).

$C_{12}H_8OS$ (200.26) Ber. C 71.97 H 4.02 S 16.01 Gef. C 71.69 H 3.87 S 15.94.

2-Methyl-5-ethoxy-naptho(1.8-bc)thiolium-tetrafluoroborat (6)

Eine Lösung von 0.4 g (2 mmol) *(5)* und 0.4 g (2 mmol) Triethyloxonium-tetrafluoroborat wird bei 35°C in 10 ml wasserfreiem 1.2-Dichlorethan etwa 20 min. gerührt - bis zur Grünfärbung. Das Salz wird mit Ether gefällt und nach Entfernung des Ethers sofort in wasserfreiem Acetonitril gelöst; die grüne Lösung ist bei Kälte und Dunkelheit etwa 2 Wochen beständig, *(6)* konnte selbst jedoch nicht isoliert werden. ^{1}H-NMR (90 MHz, D_3C-CN): δ (ppm) = 8.21 (d, H^3, I = 9 Hz), 7.65 (d, H^4, I = 9 Hz), 7.93 (d, H^6, I = 8 Hz), 8.15 (t, H^7, I = 8 Hz), 8.62 (d, H^8, I = 8 Hz), 3.26 (s, α -CH_3-), 4.32 (q, -OCH_2), 1.62 (t, -O-CH_2-CH_3).

5-Ethoxy-2-methylen-naptho(1.8-b-c) triophen (6a)

Zu einer Lösung von 0.46 g (2 mmol) *(6)* in 5 ml wasserfreiem Acetonitril werden 0.22 g (4 mmol) Anilin getropft; bei Zugabe von einigen Tropfen Ethyl-diisopropylamin («Hünig-Base») schlägt die Farbe in orange um und nach Entfernen des Lösungs mittels sowie angeschlossener säulenchromatographischer Reinigung - «Kieselgel 60 Merck» - mit Ethanol als Elutions-mittel werden gelbe Kristalle isoliert (aus Ethanol). Ausb. 150 mg (32%); Schmp. 123°C.

$C_{14}H_{12}SO$ (228.36) Ber. C 73.65 H 5.30 S 14.04
Gef. C 73.27 H 5.39 S 13.87

IR (KBr): 1620 cm^{-1} (C=CH_2). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 6.96 (d, α -CH_2-), 7.63 - 8.42 (m, H^3, H^6, H^7, H^8), 6.48 (s, H^4), 4.11 (q, -O-CH_2-), 1.41 (t -CH_3). MS (100 eV, 120°C): m/e (%) = 228 (100, M^+), 202 (35), 170 (84), 126 (93).

2-Methyl-naphtho(1.8-bc)thiophen-5-(m-chlor-phenylimin) (7a)

Zu einer Lösung von 400 mg (2 mmol) *(5)*, 307 mg (2 mmol) m-Chlorphenylisocyanat wird eine katalytische Menge von $AlCl_3$ gegeben, und 24 h unter Rückfluß erhitzt. Nach Entfernen des Lösungs-mittels wird der Rückstand über eine Kieselgelsäule - Kieselgel 60 «Merck» - mit Benzol als Lösungsmittel chromatographiert. Gelbe Kristalle (aus Benzol/Petrolether). Ausb. 112 mg (18%); Schmp. 167°C.

$C_{18}H_{12}CINS$ (309.8) Ber. C 69.78 H 3.90 N 4.52 S 10.35
Gef. C 69.24 H 3.46 N 4.59 S 10.14

IR (KBr): 1590, 1540 (C=N). ^{1}H-NMR (90 MHz, $CDCl_3$): α (ppm) = 2.73 (s, α -CH_3), 7.14 - 8.45 (m, H^3, H^7, H^8), 6.88 (d, H^4) 6.89 (d, H^6). MS (100 eV, 165°C): m/e (%) = 309.5 (74, M^+), 274 (84), 199 (100), 172 (31), 171 (97), 157 (38), 146 (25), 132 (29), 114 (11), 113 (18), 100 (20), 87 (8), 63 (10).

2-Methyl-naphtho(1.8-bc) thiophen-5-phenylimin (7b)

Eine Lösung von 400 mg (2 mmol) *(5)* in 15 ml Ethanol und 100 mg (2 mmol) Anilin wird mit 10 Tropfen 60 proz. Perchlorsäure versetzt und 2 h unter Rückfluß zum Sieden erhitzt; es werden einige Tropfen Ethyldiisopropylamin zugegeben, die Farbe der Reaktionslösung schlägt von rot nach orange um. Nach Entfernen des Lösungsmittels wird der Rückstand über Kieselgel 60 «Merck» chromatographiert - Benzol als Elutionsmittel; gelbliche Kristalle (aus Benzol/Petrolether). Ausb. 120 mg (22%); Schmp. 160°C.

$C_{18}H_{13}NS$ (275.38) Ber. C 78.51 H 4.75 N 5.09 S 11.64
Gef. C 78.31 H 4.56 N 4.89 S 11.19

IR (KBr): 1650 cm^{-1} (C=N). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 2.61 (s, α- CH_3), 7.34 - 7.65 (m, H^3, H^7, H^9), 6.82 (d, H^4), 6.54 (d, H^6). MS (100 eV, 145°C): m/e (%) = 275 (64, M^+), 199 (78), 172 (23), 171 (100), 158 (69), 157 (24), 146 (73), 132 (45), 114 (6), 113 (34), 100 (18), 87 (9), 63 (14).

2-Methyl-naphtho(1.8-bc)thiophen-5-(p-toluolsulfonylhydrazon) (7c)

Zu einer Lösung von 400 mg (2 mmol) *(5)*, 0.19 g (2 mmol) p-Toluolsulfonsäurehydrazid in 20 ml Ethanol wird mit 10 Tropfen konz. Salzsäure versetzt und 1 h unter Rückfluß zum Sieden erhitzt. Der abgesaugte Niederschlag wird mit Wasser gewaschen; dunkelgelbe Kristalle (aus Ethanol). Ausb. 400 mg (55%); Schmp. 189°C.

$C_{19}H_{16}N_2O_2S_2$ (368.5) Ber. C 61.93 H 4.91 N 7.60 S 17.40
Gef. C 61.08 H 4.72 N 7.25 S 17.18

IR (KBr): 1580, 1530 cm^{-1} (C=N). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 2.55 (s, α -CH_3), 7.16 - 7.52 (m, H^3, H^7, H^8), 6.46 (d, H^4), 6.43 (d, H^6). MS (100 eV, 85°C): m/e (%) = 368 (82, M^+), 278 (97), 214 (74), 186 (69), 172 (12), 171 (100), 158 (52), 157 (23), 146 (73), 132 (59), 114 (19), 113 (44), 87 (14), 63 (22).

2.2'-Methyl-naphtho (1.8-bc) thiophen-5.5'-azin (8)

Aus 400 mg (2 mmol) *(5)*, *64* mg (2 mmol) Hydrazin (100 proz.) in 59 ml wasserfreiem Ethanol und 5 Tropfen konz. Salzsäure analog *(7c)*. Ausb. 634 mg (80%); Schmp. 309°C.

$C_{24}H_{16}N_2S_2$ (396.5) Ber. C 72.69 H 4.07 N 7.07 S 16.17
Gef. C 72.59 H 3.86 N 6.97 S 16.04

IR (KBr): 1575, 1540 cm^{-1} (C=N). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 2.59 (s, α -CH_3), 7.41 - 7.98 (m, H^3, H^7, H^8), 6.42 (d, H^4), 6.57 (d, H^6). MS

(100 eV, 450°C): m/e (%) = 396 (44, M^+), 199 (73), 172 (23), 171 (100), 158 (74), 157 (27), 149 (69), 132 (50), 114 (11), 113 (39), 87 (14), 63 (21).

2-Methyl-naphtho(1.8-bc)thiophen-5-(pyrimidin-2',6'-dion-4'-thion-5'-yliden) (9)

400 mg (2 mmol) *(5)* und 340 mg (2 mmol) Thiobarbitursäure werden in einer Mischung aus 8 ml Acetanhydrid und 4 ml Eisessig 10 min unter Rückfluß zum Sieden erhitzt, nach dem Erkalten der Niederschlag abgesaugt. Blau-grüne Nadeln (aus Essigsäure); Ausb. 460 mg (70%); Schmp. 317°C.

$C_{16}H_{10}N_2O_2S_2$ (326.4) Ber. C 66.24 H 3.09 N 8.98 S 19.64
Gef. C 66.19 H 3.04 N 8.13 S 19.87

IR (KBr): 1695, 1650 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 2.45 (s, α -CH_3), 7.39 - 7.99 (m, H^3, H^7, H^8), 8.23 (d, H^4), 8.25 (d, H^6). MS (100 eV, 190°C): m/e (%) = 326 (65, M^+), 266 (34), 239 (45), 225 (39), 198 (56), 172 (24), 171 (100), 158 (58), 157 (23), 146 (65), 132 (49), 114 (23), 113 (48), 87 (24), 63 (23).

2-Methyl-naphtho(1.8-bc) thiophen-5-dicyanmethylen (10a)

Eine Lösung von 400 mg (2 mmol) *(5)* und 67 mg (2 mmol) Malonsäuredinitril in 10 ml Acetanhydrid wird 24 h auf 100°C erhitzt. Das Lösungsmittel wird entfernt, der Rückstand säulenchromatographisch gereinigt über Kieselgel 60 «Merck» – Benzol als Elutionsmittel: dunkelrote Kristalle (aus Benzol). Ausb. 60 mg (12%); Schmp. 266°C.

$C_{15}H_8N_2S$ (248.3) Ber. C 72.55 H 3.24 N 11.28 S 12.91
Gef. C 72.21 H 3.32 N 11.07 S 12.45

IR (KBr): 2200 (C≡N), 1570 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 270 (s, α -CH_3), 7.68 - 8.01 (m, H^3, H^7, H^8), 8.21 (d, H^4). MS (100 eV, 160°C): m/e (%) = 248 (45, M^+), 223 (39), 198 (66), 172 (34), 171 (100), 158 (69), 157 (26), 146 (64), 132 (48), 114 (8), 113 (37), 87 (14), 63 (18).

2-Methyl-naphtho(1.8-bc)thiophen-5-methoxycarbonyl-cyanomethylen (10b)

Aus 400 mg (2 mmol) *(5)*, 100 mg (2 mmol) Cyanessigsäuremethylester analog *(10a)*. Rotviolette Kristalle (aus Benzol/Petrolether) Ausb. 150 mg (27%); Schmp. 200°C.

$C_{16}H_{11}NO_2S$ (281.3) Ber. C 68.31 H 3.95 N 4.98 S 11.40
Gef. C 68.12 H 3.72 N 5.06 S 10.97

IR (KBr): 2180 cm^{-1} (C≡N). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 2.80 (s, α -CH_3), 7.44 - 7.84 (m, H^3, H^7, H^8), 7.79 (d, H^4), 8.42 (d, H^6). MS (100 eV, 160°C): m/e (%) = 281 (63, M^+), 267 (42), 250 (52), 225 (23), 223 (33), 198 (56), 172 (19), 171 (100), 158 (65), 157 (15), 146 (56), 132 (54), 114 (10), 113 (42), 87 (24), 63 (15).

2-Methyl-naptho(1.8-bc)thiophen-5-diphenyliden (10c)

Zu einer Lösung von 400 mg (2 mmol) *(5)* in 10 ml wasserfreiem Benzol werden unter N_2-Atmosphäre 400 mg (2 mmol) Diphenylketen gegeben, 1 h unter Rückfluß zum Sieden erhitzt; anschließend wird das Lösungsmittel

entfernt, der Rückstand säulenchromatographisch - Kieselgel 60 «Merck», Benzol als Lösungsmittel - gereinigt. Gelbe Nadeln (aus n-Hexan). Ausb. 640 mg (91%); Schmp. 153°C.

$C_{25}H_{18}S$ (350.5) Ber. C 85.68 H 5.18 S 9.15
Gef. C 85.74 H 5.06 S 8.89

IR (KBr): 1590, 1540 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 2.78 (s, α -CH_3), 7.16 - 7.92 (m, H^3, H^7, H^8), 6.97 (d, H^4), 7.12 (d, H^6). MS (100 eV, 155°C): m/e (%) = 350 (76, M^+), 274 (69), 198 (92), 172 (40), 171 (100), 158 (84), 157 (24), 146 (56), 132 (42), 114 (23), 113 (32), 110 (22), 87 (17), 63 (13).

2-Methyl-naphtho(1.8-bc)thiophen-5-(t-butyl-cyanyliden) (10d)

Aus 400 mg (2 mmol) *(5)* in 10 ml wasserfreiem Benzol, 246 mg (2 mmol) t-Butyl-cyano-keten unter N_2-Atmosphäre analog *(10c)*. Ausb. 480 mg (87%); Schmp. 176°C.

$C_{18}H_{17}NS$ (279.4) Ber. C 77.38 H 6.13 N 5.01 S 11.47
Gef. C 77.29 H 5.89 N 4.86 S 11.23

IR (KBr): 2120 (C≡N), 1580 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 2.84 (s, α -CH_3), 7.56 - 8.12 (m, H^3, H^7, H^8), 6.83, 7.22 (jeweils d, H^4), 7.86, 6.81 (jeweils d, H^6). MS (100 eV, 74°C): m/e (%) = 279 (81, M^+), 223 (37), 198 (94), 172 (23), 171 (100), 158 (70), 157 (23), 146 (53), 145 (41), 132 (40), 114 (21), 113 (24), 87 (13), 63 (9).

2-Methyl-naphtho(1.8-bc) thiophen-5-fluorenyliden (11)

Aus 400 mg (2 mmol) *(5)* und 390 mg (2 mmol) Fluorenketen unter N_2-Atmosphäre analog *(10c)*. Dunkelblaue Kristalle (aus n-Hexan); Ausb. 180 mg (26%); Schmp. 188°C.

$C_{25}H_{16}S$ (384.4) Ber. C 86.18 H 4.63 S 9.20
$C_{25}H_{16}S$ (348.4) Gef. C 85.81 H 5.01 S 9.04

IR (KBr): 1560, 1510 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 2.95 (s, α -CH_3), 7.38 - 7.93 (m, H^3, H^7, H^8), 7.26 (d, H^4), 7.45 (d, H^6). MS (100 eV, 140°C): m/e (%) = 348 (88, M^+), 198 (94), 172 (16), 171 (100), 158 (39), 157 (35), 146 (68), 132 (67), 114 (28), 113 (49), 87 (21), 63 (17).

2-Methyl-naphtho(1.8-bc)thiophen-5-(2', 3', 4', 5'-tetrachlorocyclopentadienyliden) (12a)

Aus 400 mg (2 mmol) *(5)*, 200 mg (2 mmol) 2,3,4,5-Tetrachlorocyclopentadien in 10 ml Acetanhydrid analog *(9)*. Violette Kristalle; Ausb. 140 mg (18%); Schmp. 146°C.

$C_{17}H_8Cl_4S$ (386.1) Ber. C 52.88 H 2.09 S 8.30
Gef. C 52.42 H 2.31 S 8.17

IR (KBr): 1580, 1530 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 2.76 (s, α -CH_3), 7.42 - 8.11 (m, H^3, H^7, H^8), 8.13 (d, H^4), 8.22 (d, H^6). MS (100 eV, 160°C): m/e (%) = 386 (63, M^+), 351 (46), 315 (45), 280 (60), 244 (55), 198 (82), 172 (17), 171 (100), 158 (25), 157 (21), 146 (73), 132 (55), 114 (16), 113 (53), 87 (9), 63 (9).

2-Methyl-naphtho(1.8-bc) thiophen-5-(2', 3', 4', 5'-tetracyanocyclopentadienyliden) (12b)

Aus 400 mg (2 mmol) *(5)*, 100 mg (2 mmol) 2.3.4.5-Tetracyanocyclopentadien in 10 ml Acetanhydrid analog *(9)*; dunkelblaue Kristalle (aus n-Hexan). Ausb. 160 mg (23%); Schmp. 178°C.

$C_{21}H_8N_4S$ (348.4) Ber. C 72.4 H 2.31 N 16.08 S 9.20
Gef. C 72.19 H 2.14 N 15.89 S 9.10

IR (KBr): 2210 (C≡N), 1575, 1525 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.17 - 8.17 (m, H^3, H^7, H^8), 8.62 (d, H^4), 8.73 (d, H^6), 2.83 (s, α -CH_3). MS (100 eV, 270°C): m/e (%) = 348 (75, M^+), 322 (45), 296 (68), 270 (59), 244 (85), 198 (92), 172 (36), 171 (100), 146 (70), 132 (64), 114 (21), 113 (47), 87 (20), 63 (13).

2-Methylnaphtho(1.8-bc)thiophen-5-(2', 3', 4', 5'-tetramethoxy-carbonyl-cyclopentadienyliden) (12c)

Zu einer Lösung von 400 mg (2 mmol) *(5)* in 10 ml Acetonitril werden 470 mg (2 mmol) 2,3,4,5-Tetramethoxycarbonyl-cyclopentadien[11] gegeben, anschließend 6 h unter Rückfluß erhitzt, der nach dem Erkalten enstandene Niederschlag wird säulenchromatographisch - über Kieselgel 60 «Merck AG», Benzol als Elutionsmittel - gereinigt; blauschwarze Kristalle (aus Methylenchlorid). Ausb. 180 mg (19%); Schmp. 194°C.

$C_{25}H_{20}O_8S$ (480.5) Ber. C 62.49 H 4.20 S 6.67
Gef. C 62.65 H 3.89 S 6.45

IR (KBr): 1720 (C=O), 1575, 1530 cm^{-1} (C=C), ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.41 - 8.02 (m, H^3, H^7, H^8), 8.02 (d, H^4), 8.12 (d, H^6), 2.67 (s, a -CH_3). MS (100 eV, 250°C): m/e (%) = 480 (75, M^+), 466 (46), 424 (49), 421 (69), 379 (28), 362 (63), 334 (48), 303 (75), 289 (42), 244 (57), 198 (51), 198 (85), 172 (15), 171 (100), 158 (69), 157 (24), 146 (67), 132 (63), 114 (14), 113 (63), 87 (24), 63 (16).

2-Methyl-naphtho(1.8-bc)thiophen-5-(2', 3', 4', 5'-tetraphenylcyclopentadienyliden) (12d)

Zu einer Lösung von 1.0 g (5 mmol) *(5)* und 1.98 g (5 mmol) 2,3,4,5-Tetraphenyl-1-diazo-cyclopentadien in Xylol/Nitrobenzol wird eine katalytische Menge $AlCl_3$ gegeben, 6 h unter Rückfluß erhitzt; nach dem Erkalten wird der Niederschlag säulenchromatographisch - Kieselgel 60 «Merck AG», Benzol/CCl_4 als Elutionsmittel - gereinigt; schwarze, glänzende Kristalle (aus n-Hexan/Benzol). Ausb. 70 mg (6%); Schmp. 239°C.

$C_{41}H_{28}S$ (552.7) Ber. C 89.09 H 5.11 S 5.80
Gef. C 89.11 H 5.32 S 5.66

IR (KBr): 1570, 1520 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.19 - 8.60 (m, H^3, H^7, H^8), 8.02 (d, H^4), 7.42 (d, H^6), 2.78 (s, α -CH_3). MS (100 eV, 170°C): m/e (%) = 552 (78, M^+), 475 (70), 398 (68), 321 (73), 244 (75), 198 (86), 172 (27), 171 (100), 158 (74), 157 (16), 146 (52), 132 (47), 114 (23), 213 (49), 87 (21), 63 (14).

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Summary

Heterocyclic 12-π-and 14-π-systems, 27:
Syntheses and reactivities of new thia-pseudophenalenone-derivatives

The reaction of 2-methyl-benzo(b)thiophene *(1)* with α-chloro-dimethyl-ether yields 2-methyl-3-chloromethylbenzo(b)thiophene *(2).* Compound *(2)* is converted according to known methods to the acid *(3),* which after cyclisation and oxidation yields *(4)* and *(5).* The alkylation of *(5)* with «Meerwein -salt» yields the thia-pseudophenaleniumtetrafluoroborate *(6),* which in the presence of base after rearomatisation yields *(6a).* From the reaction of *(5)* with the corresponding N-nucleophiles we obtained the derivatives *(7a-7e)* and *(8).* Similarly reaction of *(5)* with C-nucleophiles yielded the thia-pseudophenafulvanes *(9)* and *(10a-10d).* In addition, it was possible to synthetise the thia-pseudophenafulvelenes *(11)* and *(12a-12d).* The structure of all compounds was determined by elementar analysis and spectroscopic methods.

Περίληψη

Ἑτεροκυκλικὰ 12-π καὶ 14-π- συστήματα.
Σύνθεση καὶ ἰδιότητες νέων θεια-ψευδο-φαιναλενονικῶν παραγώγων.

Ἀντίδραση τοῦ 2-μεθυλο-βενζο(b)θειαφαίνιου *(1)* μὲ α-

χλωροδιμεθυλαιθέρα δίνει τὸ 2-μεθυλο-3-χλωρομεθυλο-βενζο(b)θειοφαίνιο *(2)*, τὸ ὁποῖο μὲ γνωστὲς μεθόδους μετατρέπεται ἀρχικὰ στὸ 3(2-μεθυλο-βενζο(b)θειενυλο)προπιονικὸ ὀξύ *(3)* καὶ περαιτέρω μέ κυκλοποίηση καὶ ὀξείδωση στὰ *4* καὶ *5*.

Ἀλκυλίωση τοῦ 2-μεθυλο-5-οξο-5H-ναφθο(1.8-bc)θειοφαίνιου *(5)* μὲ ἅλας MEERWEIN (τετραφθοριοβορικὸ τριαιθυλοξώνιο) δίνει τὸ τετραφθοριοβορικὸ ἅλας τοῦ 2-μεθυλο-5-αιθοξυ-ναφθο(1.8-bc)θειολίου *(6)*, ἀπὸ τὸ ὁποῖο μὲ ἐπαναρωματοποίηση μὲ ἐπίδραση βάσης λαμβάνεται τὸ 5-αιθοξυ-2-μεθυλενο-ναφθο(1.8-bc)θειοφαίνιο *(6a)*.

Ἡ ἕνωση *5* δίνει μὲ πυρηνόφιλα ἀντιδραστήρια τὰ παράγωγα *7a-7c*, *8*. *Ἀντίδραση τοῦ 5* μὲ ἑνώσεις μὲ ἐνεργὸ μεθυλενικὴ ὁμάδα δίνει τὰ θεια-ψευδοφαινοφουλβένια *9*, *10a-10*d. Παρασκευάστηκαν ἐπίσης τὰ θεια-πεντα-ψευδοφαιναφουλβαλένια *11* καὶ *12a-12d*.

Μελετήθηκαν τὰ φασματοκοπικὰ δεδομένα IR, NMR καὶ μαζῶν ὅλων τῶν νέων ἑνώσεων. Ἐξάγεται τὸ συμπέρασμα ὅτι στὴ βασικὴ ἠλεκτρονικὴ κατάσταση τῆς ἑνώσεως *5* συμμετέχει κατὰ μεγάλο ποσοστὸ ἡ διπολικὴ ὁριακὴ δομή.

Chimika Chronika, New Series, 9, 91-110 (1980)

HETEROCYCLISCHE 12-π- UND 14-π-SYSTEME, 38. MITTEILUNG. UNTERSUCHUNGEN ZUR SYNTHESE UND ZUM REAKTIONSVERHALTEN NEUER SELENA-PSEUDO-PHENALENON-DERIVATE

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Zusammenfassung

Die Reaktion von diazotierter Anthranilsäure *(1)* mit Na_2Se_2 führt zunächst zur «Diselenosalicylsäure» *(2)*, durch Reduktion derselben zur o-Carboxyphenyl-seleno-essigsäure *(3)*, aus welcher der Dimethylester *4*, unter den Bedingungen einer Dieckmann - Kondensation der α-Ketocarbonsäureester *(5)*, nach Methylierung mit CH_3I zur Verbindung *(6)*, hieraus nach Verseifung und anschliessender Decarboxylierung zu *(7)*, nach Reduktion zum Alkohol *8* und nach Wasserabspaltung zum 2-Methylbenzo (b) selenophen *(9)*. Aus *(9)* konnte mit Hilfe von α-Chlor-dimethylether das Chlormethylderivat *(10)*, anschließend nach bekannten Methoden die Propionsäure *(11)*, durch Cyclisierung das Keton *(12)* und durch Oxidation schließlich das Selena-pseudophenalenon-5 erhalten werden, das leicht mit «Meerweinsalz» zum Kation *(14)* — dem Selenapseudophenaleniumtetrafluoroborat — alkyliert wurde. Aus *(9)* und Zimtsäurechlorid in Gegenwart von $AlCl_3$ wurde das isomere Keton *(16)*, durch Alkylierung desselben das Kation *(17)* synthetisiert. Aus *(13)* wurden die Imino-selenapseudophenalenon- derivate *(18a)* - *(18c)* sowie das Azin *(19)* zugänglich gemacht, aus dem Keton *(16)* in entsprechender Weise die Imino-derivate *(20a)* - *(20c)*, *(21)* und aus *(16)* mit C-Nucleophilen die Selenapseudophenafulvene *(22)*, *(23)*, *(24a)* - *(24b)*; darüberhinaus reagierte *(16)* zu den Selena-penta-pseudophenafulvalenen *(25)* und *(26a)* - *(26d)*, in analoger Weise das Keton *(13)* zu den Selenapentapseudophenafulvalenen *(29)*, *(30a)* - *(30d)*. Wurde das Selenapseudophenalenon-5 *(13)* mit C-Nucleophilen zur Reaktion gebracht, so resultierten die Selenapseudophenafulvene *(27a)* - *(27c)* sowie *(28)*; die Eliminierung jeweils eines Protons aus den Kationen *(14)* und *(17)* führte zu den Verbindungen *(31)* und *(32)*.

Schlüsselwörter: *Heterocyclishe 12-π-Electronesysteme; heterocyclische 14-π-Elektronensysteme; 2-Methyl-benzo(b)selenophen; 2-Methyl-5-oxo-5H-naphtho(1.8-bc)selenophen; 2-Methyl-5-ethoxy-naphtho (1.8-bc) selenoniumkation; 2-Methyl-3-oxo-3H-naphtho (1.8-bc) selenophen; 2-Methyl-2-ethoxy-naphtho (1.8-bc) selenoniumkation; Reaktion der Selena-pseudo-phenalenone mit N- und C-Nukleophilen; Selena-penta-pseudo-phena-fulvalene; 3-bzw. 5-Ethoxy-2-methylen-naphtho (1.8-bc) selenophen.*

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Einführung

In Fortführung unserer Untersuchungen *(2 - 11)* über Hetero-pseudo-phenalenone, über Hetero-penta-pseudophenafulvalene und über die Hetero-pseudo-phenafulvene beschäftigten wir uns auch mit den entsprechenden Selena-pseudo-phenalen-derivaten, also mit jenen Verbindungen, die sich vom Phenalen dadurch ableiten, dass eine Doppelbindung formal durch Selen ersetzt worden ist. Die Selena-pseudo-phenalenone enthielten in α-Stellung eine CH_3-gruppe und in Position 5 bzw. 3 die Carbonylgruppe. Es wurden die Derivate der Reaktionen mit N-Nukleophilen, C-Nukleophilen, sowie die Selena-penta-pseudophenafulvalene hergestellt. Es konnte gezeigt werden, daß die Selena-pseudophenalenone im elektronischen Grundzustand in bedeutendem Maße die zwitterionische dipolare Grenzstruktur einnehmen.

Ausgehend von diazotierter Anthranilsäure *(1)* wurde diese mit Natrium-selenid zum Di-natriumsalz der Diphenyl-diselenid-di-o-carbonsäure-«Diselenosalicylsäure» - umgesetzt und die freie Säure *(2)* mit Hilfe von Salzsäure ausgefällt. Durch Reduktion von *(2)* mit Zinkstaub und daran angeschlossener Reaktion mit Natriumchloracetat wird o-Carboxyphenyl-seleno-essigsäure *(3)* erhalten, daraus der Dimethylester *(4)*, unter den Bedingungen einer Dieckmann- Kondensation (Natriumethoxylat) der bicyclische α-Ketocarbonsäureester *(5)*, der mit einer äquimolaren Menge Methyliodid zum (2.3-Dihydro-)-2-ethoxycarbonyl-2-methyl-3-oxobenzo(b) selenophen *(6)* methyliert wird[14]. *(6)* wird zur Säure hydrolisiert, anschließend diese zu *(7)* decarboxyliert, das Keton *(7)* zum Alkohol *(8)* reduziert und daraus H_2O unter Bildung von 2-Methylbenzo(b) selenophen *(9)* eliminert. *(9)* wird mit Hilfe von α-Chlor-dimethylether in *(10)* überführt[15], nach bekannten Methoden in die Säure *(11)*[15] und schließlich das Säurechlorid von *(11)* zum tricyclischen Keton cyclisiert, das seinerseits zum Selenapseudophenalenon *(13)* oxidiert wird[16]. Im IR-Spektrum von *(13)* ist eine niederfrequente Carbonylvalenzschwingung bei 1640 cm^{-1} zu beobachten, die sicherlich auf einen hohen Anteil der zwitterionischen, dipolaren Grenzstruktur am electronischen Grundzustand zuruckzuführen sein dürte. Erwartungsgemäß konnte *(13)* durch «Meerweinsalz» - dem Triethyloxoniumtetrafluoroborat - zum 2-Methyl-5-ethoxy-selenapseudophenaleniumtrafluoroborat *(14)* alkyliert werden.

Das zu *(13)* in Stellung 3 isomere Selenapseudophenalenon-3 konnte nach unseren Erfahrungen[9] aus *(9)* und Zimtsäurechlorid in Gegenwart von Aluminiumchlorid gewonnen werden, dessen IR-Spektrum ebenfalls eine sehr niederfrequente Carbonyl-valenzchwingung bei 1615 cm^{-1} aufweist und daher das 2-Methyl-3-ethoxy-selenapseudophenaleniumtetrafluoroborat *(17)* auch leicht durch Alkylierung an der Carbonylgruppe zu synthetisieren war.[1,3,5,6]

Wurde das Keton *(13)* mit m-Chlorphenylisocyanat in Gegenwart von katalytischen Mengen an $AlCl_3$ behandelt, so bildete sich unter Eliminierung von CO_2 das Imino-selena-pseudophenalenon- derivat *(18a)*, aus *(13)* und Anilin in Anwesenheit katalytischer Mengen Säure die Verbindung *(18b)* sowie in analoger Weise mit p-Tosylhydrazin das Hydrazonderivat *(18c)*, mit

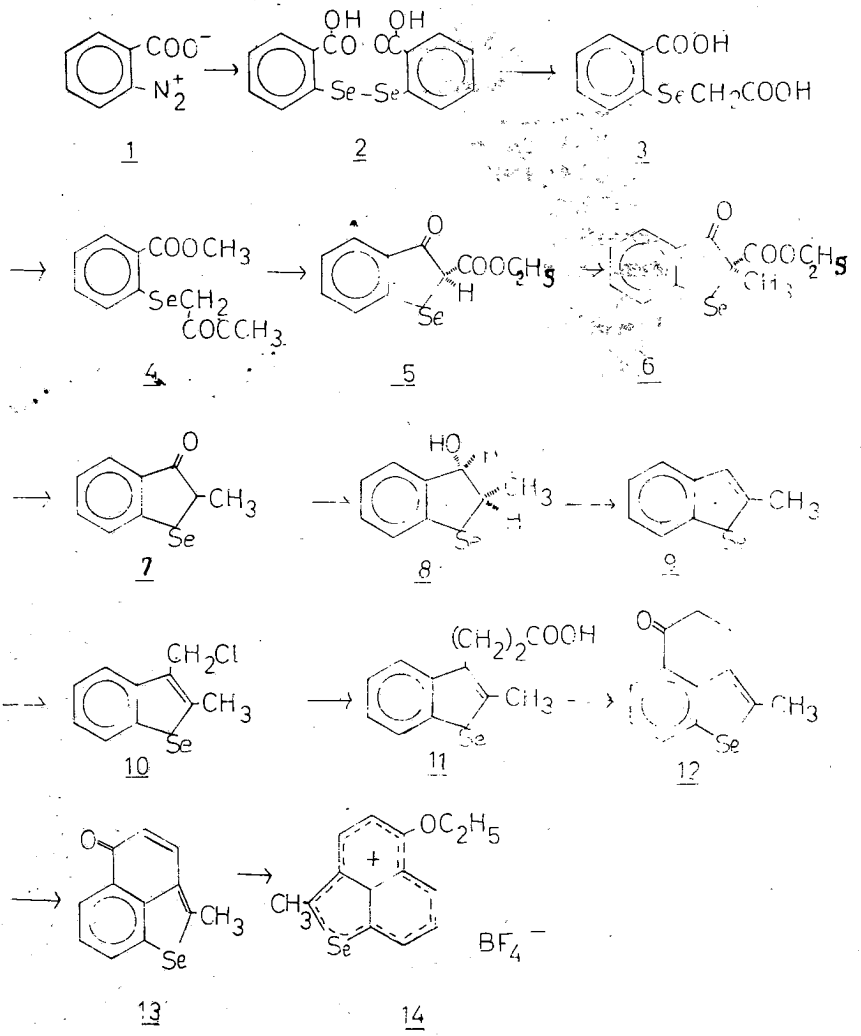


Abb 1

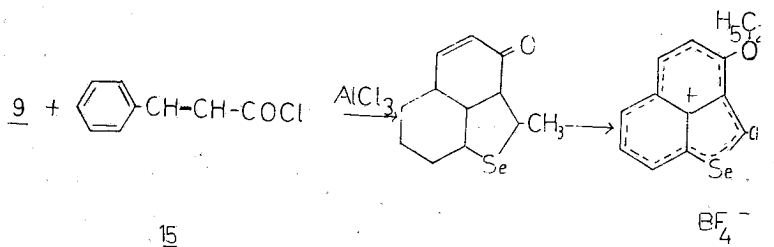
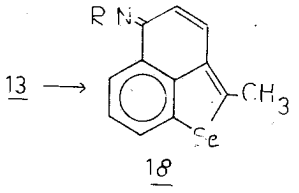


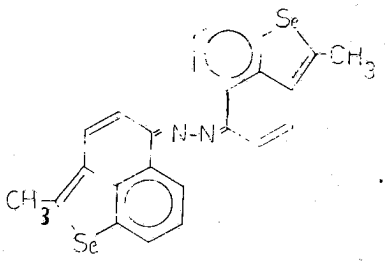
Abb 2

Hydrazin selbst das blauviolette Azin *(19)*. Ein ähnliches Reaktionsverhalten zeigte auch das zu *(13)* isomere Keton *(16)*, welches mit N-Nucleophilen die 3-Imino-selena-pseudophenalenon-derivate *(20a)* - *(20c)* sowie das violette Azin *(21)* lieferte und mit entsprechenden C-Nucleophilen die Selena-pseudophenafulvene *(22)*, *(23)*, sowie *(24a)* - *(24b)*. In der Verbindung *(24b)* ist beim Isomeren mit $R^1 = C(CH_3)_3$ die α-Methylgruppe durch die Cyanogruppe nach tieferem Feld verschoben; H^4 zeigt sich dagegen bei relativ hohem Feld. Beim anderen Isomeren ist H^4 aufgrund der benachbarten Cyanogruppe entschirmt, wobei α-CH_3 nach höherem Feld verschoben ist.

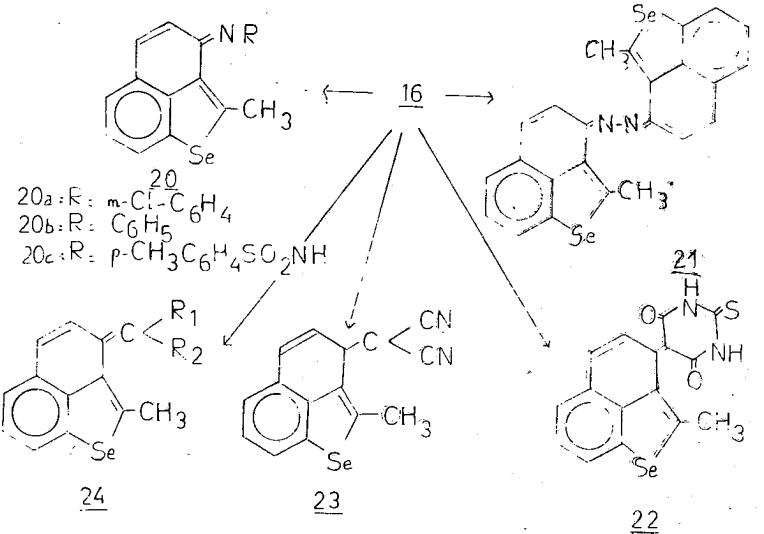


18

18a : R = m-Cl-C_6H_4
18b : R = C_6H_5
18c : R = p-CH_3-C_6H_4-SO_2NH



19



20

20a : R = m-Cl-C_6H_4
20b : R = C_6H_5
20c : R = p-$CH_3C_6H_4SO_2NH$

24 23 22

24a : $R_1 = R_2 = C_6H_5$
24b : $R_1 = C(CH_3)_3$ $R_2 = CN$
$R_1 = CN$ $R_2 = C(CH_3)_3$

Abb. 3

Als Vertreter der Selenapenta pseudophenafulvalene wurden die Fulvalene *(25), (26a)* - *(26d)* synthetisiert, ausgehend von Selenapseudophenalenon *(13)* die entsprechenden Pentapseudo-phenafulvalene *(29)* und *(30a)* - *(30d)*; in nahezu analoger Weise, wie bei *(16)*, reagierte auch das Selenapseudophenalenon-5 mit C-Nucleophilen zu den Selenap-

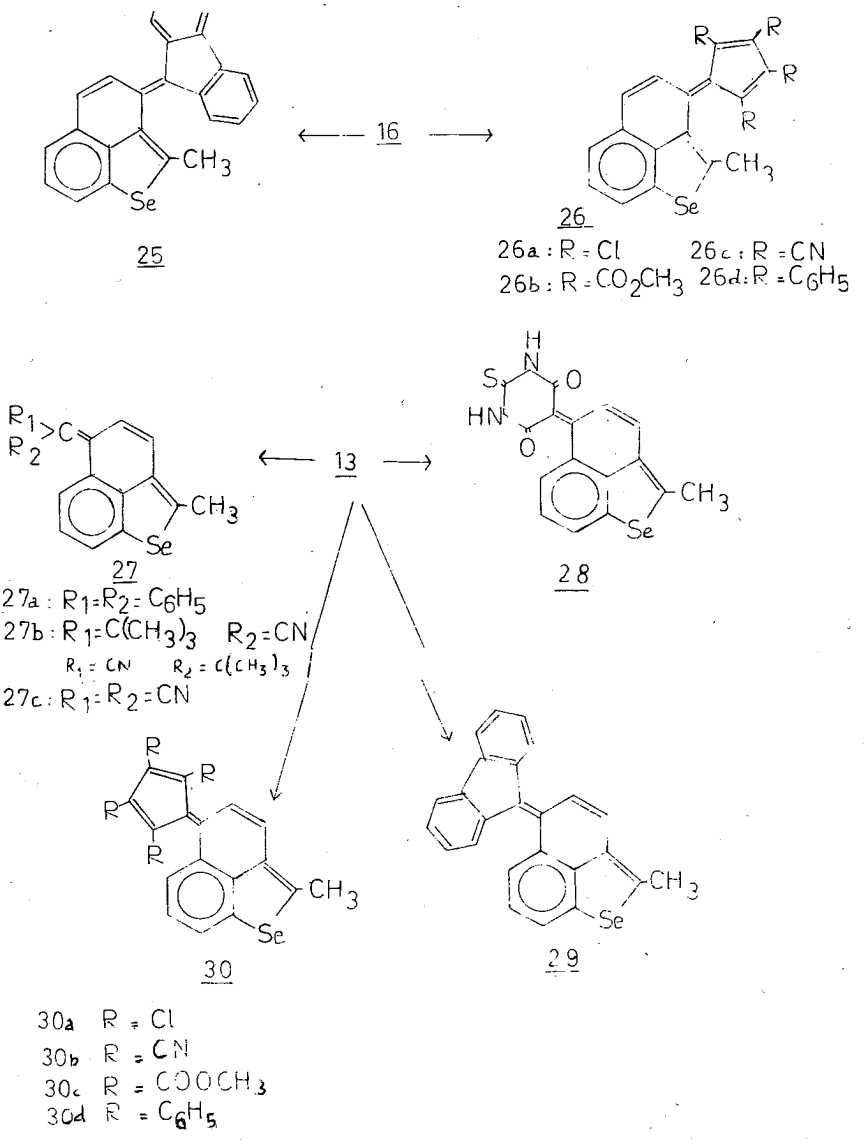


Abb. 4

seudophenafulvenen *(27a)* - *(27c)* und *(28)*. In der Verbindung *(27b)* ist beim Isomeren mit $R^1 = C(CH_3)_3$ H^6 durch die Cyanogruppe nach tieferem Feld verschoben; H^4 zeigt sich dagegen bei relativ hohem Feld. Beim anderen Isomeren liegt H^6 im Aromatenbereich; H^4 ist stark entschirmt, aufgrund der benachbarten Cyanogruppe.

Aus 2-Methyl-5-ethoxyselenapseudophenaleniumtetrafluoroborat *(14)* und 2-Methyl-3-ethoxypseudophenaleniumtetrafluoroborat *(17)* waren in Gegenwart einer Base unter Eliminierung von jeweils einem Proton und gleichzeitiger Rearomatisierung die Verbindungen *(31)* und *(32)* mit exocyclischer Doppelbindung erhalten worden; die letzteren Verbindungen erwiesen sich als sehr stabil.

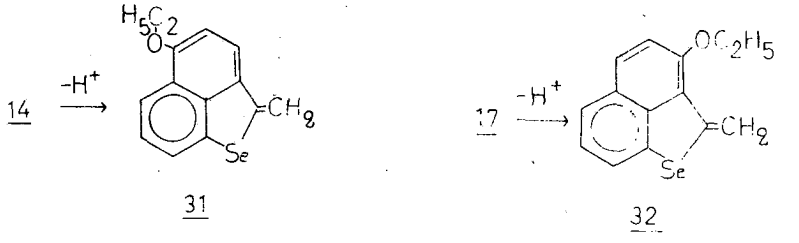


Abb. 5

Experimenteller Teil

Schmelzpunkte: nicht korrigiert, Schmelzpunktmikroskop der Fa. Reichert, Wien. — Massenspenktren: Varian MAT 311 A, dessen inverse Nier-Johnson-Geometrie die Aufnahme der DADI-Spektren ermöglichte. — ^{1}H-NMR-Spektren: Geräte Bruker HX 90 E und teilweise auch T-60-A; TMS als interner Standard. — IR-Spektren: Gerät Perkin-Elmer 177. — UV-Spektren: Gerät DMR 10 der Fa. Zeiss, Oberkochen. — Die Elementaranalysen wurden mit dem C,H,N-Analysator der Fa. Heraeus, Hanau, ausgeführt. Säulenchromatographische Trennungen und Reinigungen wurden auf einer mit Kieselgel 60 «Merck» (0.063 - 0.020 mm) beschickten Säule von 80 cm Länge und einem Innendurchmesser von 2.5 cm durchgeführt, präparative schichtchromatographische Untersuchungen auf mit Kieselgel 60 GF_{254} «Merck» bestrichenen Glasplatte (20×20 cm). Die Lösungsmittel und Reagenzien wurden nach den üblichen Methoden getrocknet und gereinigt.

Diphenyl-diselenid-di-o-carbonsäure (2):

137 g (1 mol) Anthranilsäre in 200 ml konz. Salzsäure und 130 ml Wasser werden unter Rühren auf 0°C gekühlt; 69 g (1 mol) Natriumnitrit in 200 ml Wasser werden tropfenweise unter Rühren zugegeben und die Temp. auf 0°C gehalten. Bei einer Temp. von 5°C werden langsam 140 g diazotierte Anthranilsäre *(1)* zur wässrigen Lösung von 102 g (0.5 mol) Dinatriumdiselenid gegeben, außerdem eine Lösung von 60 g NaOH in 120 ml Wasser.

Nach Entfernung des abgeschiedenen roten Selens wird die Diselenosalicylsäure *(2)* mit konz. Salzsäure gefällt und aus Eisessig unter Zusatz von Tierkohle umkristallisiert; weiße Kristalle. Ausb. 121 g (60%); Schmp. 296-297°C.

$C_{14}H_{10}O_2Se$ (320.9) Ber. C 52.40 H 3.14 Gef. C 52.31 H 3.02.

o-Carboxyphenyl-selenoessigsäure (3)

Eine Lösung von 8 g (20 mmol) Diselenosalicylsäure *(2)* in 100 ml 20proz. NaOH werden 30 min erhitzt, dazu 40 g (1.35 mol) Zinkstaub über einen Zeitraum von etwa 2 h gegeben. Die mit Zink erhitzte Lösung wird direkt in eine wässrige Lösung von 2.33 g (20 mmol) Natrium- α-Chloracetat filtriert. Nach dem Erwärmen auf 40°C bildet sich das Natriumsalz der Phenylselenoglycol-o-carbonsäure, das nach Zugabe von 7.3 g konz. Salzsäure in die Säure übergeht. Weiße Kristalle (aus Eisessig). – Schmp. 233-234°C; Ausb. 4.68 g (89%).

$C_9H_8O_4Se$ (259.0) Ber. C 41.73 H 3.11 Gef. 41.36 H 2.97.

o-Methoxy-carbonyl-phenyl-seleno-essigsäuremethylester (4)

Eine Mischung von 65.1 g (0.25 mol) *(3)*, 48.0 g (1.5 mol) Methanol und 300 ml 1.2-Dichlorethan wird über Nacht unter Rückfluß erhitzt; das Reaktionsgemisch wird mit 15proz. Natriumbikarbonatlösung gewaschen, die organische Phase abgetrennt, das Lösungsmittel entfernt und der Rückstand aus n-Hexan-Benzol umkristallisiert. Weiße Kristalle. Schmp. 169°C; Ausb. 67 g (98%).

$C_{11}H_{12}O_4Se$ (284.1) Ber. C 46.50 H 4.26 Gef. C 46.39 H 3.13.

*2.3-Dihydro-2-ethoxycarbonyl-3-oxo-benzo(b)*selenophen *(5)*

Zu einer Lösung von 1.36 g (0.2 mol) *(4)* in 20 ml Ethanol werden unter Rühren und Erwärmen 23.65 g Natriumethylat zugetropft. Nach dreistündigem Erhitzen unter Rückfluß wird 10proz. Salzsäure dazugegeben, aus Ether extrahiert - 3 mal mit je 20 ml Ether-und anschließend aus n-Hexan/Benzol umkristallisiert. Weiße Kristalle; Schmp. 89 - 90°C; Ausb. 43.1 g (88%). – $C_{11}H_{10}O_3$ (190.2) Ber. C 69.46 H 5.30 Gef. C 69.09 H 5.37 – IR (KBr): 1680 cm^{-1} (C=O). – ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 1.23, 1.15, 1.08 (t), 4.27, 4.19, 4.11, 4.04 (q).

2.3-Dihydro-2-ethoxycarbonyl-2-methyl-3-oxo-benzo(b) selenophen (6)

24.5 g (0.1 mol) 2.3-Dihydro-2-methoxy-carbonyl-3-oxo-benzo(b)-selenophen *(5)* und 28.2 g (0.2 mol) Methyliodid in 50 ml Ethanol werden 3 h lang unter Rückfluß erhitzt, mit 100 ml Wasser verdünnt und mit jeweils 20 ml Ether dreifach extrahiert; gelbe Kristalle (n-Hexan/Benzol). Schmp. 79°C; Ausb. 16.4 g (63%).

$C_{12}H_{12}O_3Se$ (283.2) Ber. C 50.88 H 4.24 Gef. C 50.58 H 4.14 – ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 1.96 (α-CH_3), 4.16 (q, -CH_2-), 1.19 (t, –CH_3), 7.02 - 7.86 (aromatische Protonen).

2.3-Dihydro-2-methyl-3-oxo-benzo(b) selenophen (7)

25.4 g (0.1 mol) *(6)* werden 6 h zur Verseifung mit 6 g (0.15 mol) wässriger NaOH-Lösung (5 proz.) erhitzt, nach dem Erkalten angesäuert und anschließend decarboxyliert; gelbe Nadeln (aus n-Hexan/Benzol). Schmp. 52°C; Ausb. 20.8 g (96%).

C_9H_8OSe (211.1) Ber. C 51.20 H 3.82 Gef. C 50.76 H 3.97.

2.3-Dihydro-2-methyl-3-hydroxy-benzo(b) selenophen (8)

Eine Lösung von 21.1 g (0.1 mol) *(7)* in 90 ml wasserfreiem Ether wird in 25.8 g (0.11 mol) $AlCl_3$ - gelöst in 50 ml wasserfreiem Ether - so zugetropft, daß der Ether mäßig siedet. Anschließend wird die Lösung 3 h unter Rückfluß erhitzt, aufgearbeitet und der Niederschlag umkristallisiert. Schmp. 49°C; Ausb. 21.1 g (97%).

C_9H_9OSe (212.1) Ber. C 51.21 H 4.28 Gef. C 51.36 H 4.23.

2-Methyl-benzo(b) selenophen (9)

Eine Lösung von 21.1 g (0.1 mol) *(8)* in 15 ml wässriger Phosphorsäure (85 proz.) wird auf 120 - 160°C unter Vakuum erhitzt und anschließend aufgearbeitet. Gelbe Kristalle; Schmp. 51°C; Ausb. 19.4 g (98%).

C_9H_8Se (195.1) Ber. C 55.40 H 4.13 Gef. C 55.29 H 3.98 – MS (70 eV, 190° C m/e (%) = 196 (67, M^+), 195 (59), 114 (20), 113 (100), 87 (13), 63 (11).

2-Methyl-3-chlormethyl-benzo(b) selenophen (10)

Zu einer Lösung von 19.5 g (0.1 mol) *(9)* in 100 ml Eisessig werden 10 g (0.125 mol) Chlormethyl-methylether in 10 ml Eisessig tropfenweise zugegeben, 24 h stehengelassen und nach Entfernung des kristallinen Niederschlags dieser aus n-Pentan umkristallisiert. Gelbe Kristalle; Schmp. 70°C; Ausb. 17 g (70%).

$C_{10}H_9ClSe$ (243.3) Ber. C 49.36 H 3.73 Gef. C 48.79 H 3.63.

3-(2-Methylbenzo(b) selenienyl-)- propionsäure (11)

20.68 g (0.085 mol) *(10)* Werden mit 13.60 g (0.085 mol) Malonsäurediethylester in 15 ml wasserfreiem Ethanol zu 2-Methyl-3- (β-carbethoxy-)- ethylbenzo(b) selenophen umgesetzt, dieses durch dreistdg. Erhitzen mit 1 N NaOH verseift, der abgetrennte und getrocknete Niederschlag 20 min lang auf 250°C erhitzt. Nach Beendigung der CO_2 - Entwicklung wird der Rückstand umkristallisiert. Weiße Nadeln (aus Petrolether 60-80); Schmp. 195°C; Ausb. 13.2 g (58%).

$C_{12}H_{12}O_2Se$ (267.18) Ber. C 53.95 H 4.53
Gef. C 54.11 H 4.62

2-Methyl-4.5-dihydro-5-oxo-5H-naphtho (1.8-bc) selenophen (12)

1. Methode: Eine Lösung von 1.94 g (7.25 mmol) *(11)* in 20 ml 1.2-Dichlorethan wird mit 5.0 ml Thionylchlorid 30 min lang unter Rückfluß erhitzt; nach Entfernung des überschüssigen $SOCl_2$ und des Lösungsmittels

wird der Rückstand in 20 ml 1.2-Dichlorethan gelöst, 20 min bei 0°C mit 1.3 g $AlCl_3$ behandelt, dann die Reaktionslösung noch 30 min bei 0°C gerührt, schließlich mit 50 ml konz. Salzsäure/ Eis (1:1) hydrolysiert. Die organische Phase wird mit kleinen Portionen 15 proz. $NaHCO_3$-Lösung, danach mit Wasser gewaschen, das Lösungmittel entfernt und der Rückstand umkristallisiert. Gelbe Kristalle (aus 1.2-Dichlorethan/n-Hexan); Schmp. 88°C; Ausb. 1.3 g (72%).

$C_{12}H_{10}O$ Se (249.2) Ber. C 57.84 H 4.05 Gef. C 57.35 H 3.89

2. Methode: 19.5 g (0.1 mol) *(9)* in 100 ml Eisessig und 12.3 g (0.1 mol) β-Methoxypropionsäurechlorid in 30 ml Eisessig werden 24 h lang bei 30°C gerührt, das Säurechlorid mit 15.9 g (0.12 mol) $AlCl_3$ in 1.2-Dichlorethan bei 0°C 20 min lang behandelt, mit 100 ml eines Gemisches aus konz. Salzsäure/Eis (1:1) hydrolysiert, die organische Phase mit 15proz. $NaHCO_3$ - Lösung gewaschen, das Lösungsmittel entfernt und der Rückstand umkristallisiert. Gelbe Nadeln (aus 1.2-Dichlorethan/n-Hexan). Ausb. 11.9 g (48%); Schmp. 88°C.

2-Methyl-5-oxo-5H-naptho (1.8-bc) selenophen (13)

0.56 g (2.25 mmol) *(12)* und 0.76 g (4.5 mmol) Triphenylmethyl-perchlorat in wasserfreiem Eisessig werden 30 min lang bei 30°C gerührt und anschließend in 15 ml Wasser gegossen, dann noch 30 ml Benzol dazugegeben, die benzolische Phase mehrfach mit jeweils 10 ml konz. Salzsäure extrahiert. Nach Abtrennung der Salzsäurephase wird diese auf 50 ml mit Wasser verdünnt, das erhaltene Produkt umkristallisiert. Gelbe Nadeln (aus 1.1-Dichlormethan, n-Hexan). Schmp. 72°C; Ausb. 11.9 g (48%).

$C_{12}H_8O$ Se (247.1) Ber. C 58.31 H 3.26 Gef. C 57.84 H 3.67 — IR (KBr): 1640 cm^{-1} (C=O); ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.17 (d, H_3, I = 10 Hz), 6.89 (d, H_4, I = 10 Hz), 2.52 (s, α-CH_3), 7.75 (d, H^6, I = 8 Hz), 7.12 (t, H^7, I = 8 Hz), 7.42 (d, H^8, I = 8 Hz). — MS (70 eV, 120°C): m/e (%) = 248 (92), 247 (80, M^+), 220 (11), 219 (71), 167 (100), 141 (26), 139 (85), 114 (22), 113 (32), 87 (9), 63 (12).

2-Methyl-5-ethoxy-naphtho (1.8-bc) selenoniumtetrafluoroborat (14)

Eine Lösung von 0.49 g (2 mmol) *(13)* und 0.4 g (2 mmol) Triethyl-oxoniumtetrafluoroborat wird bei 35°C in 10 ml wasserfreiem 1.2-Dichlorethan etwa 20 min gerührt, dann wird das Salz durch langsame Zugabe von Ether gefällt und nach Entfernen des Ethers dieses sofort in wasserfreiem Acetonitril gelöst. Die grüne Lösung ist bei etwa 0°C und in der Dunkelheit etwa 10 Tage beständig. ^{1}H-NMR (90 MHz, CD_3CN): δ (ppm) = 8.07 (d, H^3, I = 9 Hz), 7.49 (d, H^4, I = 9 Hz), 7.86 (d, H^6, I = 8 Hz), 6.14 (t, H^7, I = 8 Hz), 8.64 (d, H^8, I = 8 Hz), 3.01 (s, α-CH_3-), 4.40 (q, -O-CH_2-), 1.64 (t, -O-CH_2-CH_3).

2-Methyl-3-oxo-3H-naphtho (1.8-bc) selenophen (16)

Zu einer Lösung von 19.5 g (0.1 mol) *(9)* und 16.6 g (0.1 mol) Zimtsäurechlorid in 50 ml Benzol werden in einem Zeitraum von 25 min bei

einer Temp. von 5°C 36 g (0.27 ml) Aluminiumchlorid gegeben. Anschließend wird die Lösung 25 min unter Rückfluß erhitzt, mit 50 ml konz. Salzsäure/ Eis (1:1) hydrolysiert und die Lösung zweimal mit je 100 ml Benzol extrahiert, die vereinigten benzolischen Extrakte getrocknet und das Lösungsmittel entfernt; der Rückstand wird umkristallisiert. Gelbe Kristalle (aus n-Hexan/Benzol). Schmp. 70°C; Ausb. 17 g (60%).

$C_{12}H_8O$ Se (247.1) Ber. C 58.31 H 3.26 Gef. C 57.92 H 3.66.

IR (KBr): 1615 cm^{-1} (C=0). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 6.36 (d, H^4, I = 10 Hz), 7.30 (d, H^5, I = 10 Hz), 7.88 (d, H^6, I = 8 Hz), 7.38 (t, H^7, I = 8Hz), 7.51 (d, H^8, I = 10 Hz), 3.21 (s, α-CH_3). MS (70 eV, 80°C): m/e (%) = 248 (87), 247 (73, M^+), 220 (16), 219 (56), 167 (84), 141 (34), 139 (100), 114 (26), 113 (39), 87 (16), 63 (9).

2-Methyl-3-ethoxy-naphtho (1.8-bc) selenonium-tetrafluoroborat (17)

Die Lösung von 0.49 g (2 mmol) *(16)* und 0.4 g (2 mmol) Triethyloxoniumtetrafluoroborat wird bei 35°C in 10 ml wasserfreiem 1.2-Dichlorethan 25 min lang gerührt, die Reaktionslösung färbt sich grün und das Salz fällt nach Zugabe von Ether aus; das Salz ist jedoch nur etwa drei Tage in Lösung beständig.

^{1}H-NMR (90 MHz, CD_3CN): δ (ppm) = 7.29 (d, H^4, I = 9 Hz), 8.40 (d, H^5, I = 9 Hz), 8.22 (d, H^6, I = 8 Hz), 7.95 (t, H^7, I = Hz), 8.65 (d, H^8, I = 8 Hz), 3.05 (s, α-CH_3-), 4.78 (q, -O-CH_2-), 1.73 (t, -O-CH_2-CH_3).

2-Methyl-naphtho (1.8-bc) selenophen-5-(m-chlorphenylimin) (18a)

Eine Lösung von 0.5 g (2 mmol) *(13)*, 0.31 g (2 mmol) m-Chlorphenylisocyanat und eine katalytische Menge von Aluminiumchlorid werden 24 h unter Rückfluß erhitzt, danach abkühlen gelassen, das Lösungsmittel entfernt und der Rück stand über eine Kieselgelsäule - Kieselgel 60 «Merck» - mit Benzol als Elutionsmittel chromatographiert. Gelbe Kristalle (aus Benzol/Petrolether).

Schmp. 146°C; Ausb. 0.1 g (14%).

$C_{18}H_{12}Cl$ N Se (356.7) Ber. C 60.61 H 3.39 N 3.93
Gef. C 59.98 H 3.12 N 3.49

^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.32 - 8.63 (m, H^3, H^7, H^8), 6.83 (d, H^4), 6.80 (d, H^6), 2.51 (s, α-CH_3). MS (100 eV, 155°C): m/e (%) = 357 (69, M^+), 322 (84), 247 (94), 246 (37), 166 (68), 139 (100), 114 (34) 113 (62), 87 (9), 63 (23).

2-Methyl-naptho (1.8-bc)selenophen-5-phenylimin (18 b)

0.5 g (2 mmol) *(13)* in 15 ml Ethanol und 0.1 g (2 mmol) Anilin in 15 ml Ethanol werden mit 10 Tropfen 60 proz. Perchlor säure versetzt und 2 h unter Rückfluß zum Sieden erhitzt, und nach dem Erkalten einige Tropfen Ethyldiisopropylamin («Hünig-Base») zugegeben. Nach Entfernung des Lösungsmittels wird der Rükstand über einer Kieselgelsäule - Kieselgel 60 «Merck» - chromatographiert - Benzol als Elutionsmittel. Gelbe Kristalle (aus Benzol/Petrolether).

Schmp. 118°C; Ausb. 0.09 g (14%).

$C_{18}H_{13}N$ Se (322.3) Ber. C 67.09 H 4.07 N 4.35
Gef. C 66.45 H 3.87 N 4.29

^{1}H-NMR (90 MHz $CDCl_3$): δ (ppm) = 7.12 - 8.22 (m, H^3, H^7, H^8), 6.58 (d, H^4), 6.82 (d, H^6), 2.59 (s, α-CH_3) – MS (100 eV, 145°C): m/e (%) : 323 (74, M^+), 247 (45), 246 (49), 166 (100), 139 (56), 114 (20), 113 (42), 87 (14), 63 (9).

2-Methylnaphtho (1.8-bc) selenophen-5(p-toluylsulfonyl) – hydrazon (18 c)

Aus 0.5 g (2 mmol) *(13)* in 20 ml Ethanol und 0.2 g (2 mmol) p-Toluolsulfonsäurehydrazid analog *(18 b)*. Gelbe Kristalle; Schmp. 167°C; Ausb. 0.42 g. (50%).

$C_{19}H_{16}N_2O_2S$ Se (415.4) Ber. C 54.94 H 3.88 N 6.74
Gef. C 54.77 H 3.63 N 7.08

^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.32 - 8.31 (m, H^3, H^7, H^8), 6.25 (d, H^4), 6.03 (d, H^6), 2.61 (s, α-CH_3). MS (100 eV, 90°C): m/e (%) = 416 (76, M^+), 326 (52), 262 (45), 247 (31), 246 (52), 166 (100), 139 (67), 114 (28), 113 (46), 87 (7), 63 (8).

2.2'-Methylnaphtho(1.8.bc) selenopheno-5.5'-azin (19)

Eine Lösung von 0.5 g (2 mmol) *(13)* und 0.064 g (2 mmol) Hydrazin (100 proz.) in 15 ml wasserfreiem Ethanol wird mit 5 Tropfen konz. Salzsäure versetzt, 1 h unter Rückfluß zum Sieden erhitzt, der kristalline Niederschlag abgetrennt. Blauviolette Kristalle (aus Ethanol); Schmp. 296°C; Ausb. 0.62 g (70%).

$C_{24}H_{16}N_2Se_2$ (490.3) Ber. C 58.79 H 3.29 N 5.71
Gef. C 58.28 H 3.43 N 4.41

^{1}H-NMR (90 MHz $CDCl_3$): δ (ppm) = 7.49 - 8.45 (m, H^3, H^7, H^8), 6.12 (d, H^4), 6.42 (d, H^6), 2.63 (s, α-CH_3).

MS (100 eV, 450°C): 491 (76, M^+), 247 (28), 246 (74), 166 (92), 139 (100), 114 (28), 113 (46), 87 (9), 63 (11).

2-Methyl-naphtho(1.8-bc) selenophen-3-(m-chlorphenylimin) (20a)

Aus 0.5 g (2 mmol) *(16)* und 0.3 g (2 mmol) m-Chlor-phenylisocyanat in 10 ml trockenem Benzol analog *(18a)*. Orangefarbene Kristalle. Schmp. 152°C; Ausb. 0.04g (9%).

$C_{18}H_{12}Cl$ N Se (356.7) Ber. C 60.61 H 3.39 N 3.93
Gef. C 60.29 H 3.14 N 3.49

^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 6.98 (d, H^4), 7.08 - 7.99 (m, H^5, H^6, H^7, H^8), 186 (s, α-CH_3). MS (100 eV, 155°C): m/e (%) = 357.5 (62, M^+), 322 (78), 247 (92), 246 (32), 166 (62), 139 (100), 114 (23), 113 (65), 87 (14), 63 (12).

2-Methyl-naphtho(1.8-bc) selenophen-3-phenylimin (20 b)

Aus 0.5 g (2 mmol) *(16)* und 0.1 g (2 mmol) Anilin in 15 ml Ethanol

analog *(18 b)*. Orangefarbene Kristalle; Schmp. 143°C; Ausb. 0.11 g (17%).

$C_{18}H_{13}N$ Se (322.3) Ber. C 67.09 H 4.07 N 4.35
Gef. C 66.45 H 3.91 N 4.37

IR (KBr): 1640 cm^{-1} (C=N). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 6.81 (d, H^4), 7.09 – 8.21 (m, H^5, H^6, H^7, H^8), 1.99 (s, α-CH_3).

MS (100 eV, 150°C): m/e (%) = 323 (84, M^+), 247 (32), 246 (78), 166 (93), 139 (100), 114 (19), 113 (47), 87 (18), 63 (12).

2-Methyl-naphtho(1.8-bc)selenophen-3- (p-toluylsulfonyl)-hydrazon *(20c)*

Aus 0.5 g (2 mmol) *(16)* in 20 ml Ethanol und 0.2 g (2 mmol) p-Toluolsulfonsäurehydrazid analog *(18 b)*. Gelbe Kristalle; Schmp. 163°C; Ausb. 0.5 g (58%).

$C_{19}H_{16}N_2O_2$ S Se (415.4) Ber. C 54.94 H 3.88 N 6.74
Gef. C 54.33 H 3.38 N 6.59.

IR (KBr): 3120 (N-H), 1590, 1540 (C=N), 1340, 1151 cm^{-1} (SO_2). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 6.59 (d, H^4), 7.42 - 8.05 (m, H^5, H^6, H^7, H^8), 1.86 (s, α - CH_3). MS (100 eV, 90°C): m/e (%) = 416 (79, M^+), 326 (78), 262 (64), 247 (43), 246 (72), 166 (96), 139 (100), 114 (23), 113 (52), 87 (15), 63 (9).

2.2'-Methylnaphtho(1.8-bc) selenophen-3.3'-azin (21)

Aus 0.5 g (2 mmol) *(16)* und 0.064 g (2 mmol) Hydrazin (100 proz.) in 15 ml Ethanol analog *(19)*. Violette Kristalle; Schmp. 310°C; Ausb. 0.6 g (68%).

$C_{24}H_{16}N_2Se_2$ (490.3) Ber. C 58.79 H 3.29 N 5.71
Gef. C 58.36 H 3.10 N 5.39

IR (KBr): 1570, 1540 cm^{-1} (C=N). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 6.43 (d, H^4), 7.32 - 8.03 (m, H^5, H^6, H^7, H^8), 1.73 (s, α-CH_3). MS (100 eV, 395°C): m/e (%) = 492 (65, M^+), 247 (36), 246 (79), 166 (96), 139 (100), 114 (12), 113 (26), 87 (9), 63 (8).

2-Methyl-naphtho(1.8-bc)selenophen-3-(pyrimidin-2', 6'-dion-4'-thion-5'-yliden) (22)

0.5 g (2 mmol) *(16)* und 0.34 g (2 mmol) 2-Thio-barbitursäure werden in einer Mischung aus 8 ml Acetanhydrid und 4 ml Eisessig 10 min lang unter Rückfluß zum Sieden erhitzt, erkalten gelassen und der Niederschlag abgesaugt. Blaugrüne Nadeln (aus Eisessig); Schmp. 292°C; Ausb. 0.5 g (69%).

$C_{16}H_{10}N_2O_2S$ Se (373.3) Ber. C 51.48 H 2.70 N 7.50
Gef. C 51.35 H 2.42 N 7.36

IR (KBr): 1695, 1655 cm^{-1} (C=0). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.88 (d, H^4), 7.32 - 8.20 (m, H^5, H, H^7, H^8), 2.61 (s, α-CH_3). MS (100 eV, 145°C): 374 (62, M^+), 314 (48), 287 (39), 273 (42), 246 (65), 245 (23), 165 (92), 139 (100), 114 (23), 113 (39), 87 (9), 63 (8).

2-Methylnaphtho(1.8bc) selenophen-3-dicyanmethylen (23)

Eine Lösung von 0.5 g (2 mmol) *(16)* und 0.067 g (2 mmol) Malonsäuredinitril in 10 ml Acetanhydrid wird 24 h auf 100°C erhitzt; der Rückstand wird säulenchromatographisch (Kieselgel 60 «Merck») gereinigt - mit Benzol als Elutionsmittel; rotviolette Kristalle (aus Benzol). Schmp. 243°C; Ausb. 0.11 g (18%).

$C_{15}H_8N_2Se$ (295.2) Ber. C 61.03 H 2.73 N 9.49
Gef. C 60.46 H 2.49 N 9.22

IR (KBr): 2200 (C≡N), 1570 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.20 (d, H^4), 7.08 - 8.17 (m, H^5, H^6, H^7, H^8), 3.28 (s, α- CH_3). MS (100 eV, 160°C): m/e (%) = 296 (39, M^+), 271 (47), 246 (52), 245 (26), 165 (93), 139 (100), 114 (15), 113 (85), 87 (6), 63 (13).

2-Methyl-naptho (1.8-bc) selenophen-3-diphenyliden (24 a)

Zu einer Lösung von 0.5 g (2 mmol) *(16)* in 10 ml wasserfreiem Benzol werden unter N_2-Atmosphäre 0.4 g (2 mmol) Diphenylketen gegeben, anschließend 1 h lang unter Rückfluß erhitzt, das Lösunsgmittel entfernt und der Rückstand säulenchromatographische - Kieselgel 60 «Merck», Benzol als Elutionsmittel - gereinigt; gelborange Kristalle (aus Benzol). Schmp. 134°C; Ausb. 0.6 g (78%).

$C_{25}H_{18}Se$ (397.1) Ber. C 75.62 H 4.57
Gef. C 75.79 H 4.75

IR (KBr): 1580, 1540 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.03 (d, H^4), 7.21 - 8.54 (m, H^5, H^6, H^7, H^8), 2.75 (s, α- CH_3). MS (100 eV, 155°C): m/e (%) = 398 (74, M^+), 322 (65), 246 (23), 245 (79), 165 (94), 139 (100), 114 (16), 113 (23), 87 (8), 63 (9).

2-Methylnaphtho (1.8-bc) selenophen-3-(tert. -butyl-cyanoketen) (24 b)

0.5 g (2 mmol) *(16)* in 10 ml wasserfreiem Benzol und 0.25 g (2 mmol) tert. -Butyl-cyano-keten werden unter N_2-Atmosphäre 1 h unter Rückfluß erhitzt, der abgetrennte Niederschlag säulenchromatographisch gereinigt - Kieselgel 60 «Merck», Benzol als Elutionsmittel. Grün-gelbliche Kristalle; Schmp. 198°C; Ausb. 0.5 g (80%).

$C_{18}H_{17}N$ Se (326.3) Ber. C 66.23 H 5.25 N 4.29
Gef. C 66.17 H 5.12 N 4.38

IR (KBr): 2115 (C=N), 1580 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 6.95 (d, Isomeres α, H^4), 7.56 (d, Isomeres β, H^4), 7.28 - 8.45 (m, H^5, H^6, H^7, H^8), 2.61 (s, Isomeres α, α- CH_3), 1.97 (s, Isomeres β, α -CH_3). MS (100 eV, 74°C): m/e (%) = 327 (74, M^+), 271 (44), 246 (14), 245 (74), 165 (93), 139 (100), 114 (17), 113 (20), 87 (9), 63 (8).

2-Methyl-naphtho(1.8-bc) selenophen-3-fluorenyliden (25)

Aus 0.5 g (2 mmol) *(16)* in 10 ml wasserfreiem Benzol und 0.4 g (2 mmol) Fluorenketen analog *(24 a). Blaue Kristalle; Schmp.* 167°C; Ausb. 0.64 g (16%).

$C_{25}H_{16}Se$ (395.3) Ber. C 75.95 H 4.08 Gef. C 75.49 H 3.82. ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.62 (d, H^4), 7.22 - 8.29 (m, H^5, H^6, H^7, H^8), 2.02 (s, α- CH_3). MS (100 eV, 144°C): m/e (%) = 396 (76, M^+), 246 (62), 245 (27), 165 (84), 139 (100), 114 (32), 113 (64), 87 (8), 63 (11).

2-Methylnaphtho(1.8-bc) selenophen-3-(2', 3', 4', 5'-tetrachlorocyclopentadienyliden) (26 a)

0.5 g (2 mmol) *(16)* in 10 ml Acetanhydrid sowie 0.2 g (2 mmol) 2,3,4,5-Tetrachloro-cyclopentadien werden 6 h unter Rückfluß zum Sieden erhitzt, nach dem Erkalten der entstandene Niederschlag säulenchromatographisch - Kieselgel 60 «Merck», Benzol als Elutionsmittel - gereinigt; blaue Kristalle. Schmp. 116°C; Ausb. 0.2 g (14%).

$C_{17}H_8Cl_4Se$ (433.04) Ber. C 47.15 H 1.86
Gef. C 46.83 H 1.64

IR (KBr): 1575, 1510 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.21 (d, H^4), 7.35 - 8.13 (m, H^5, H^6, H^7, H^8), 2.89 (s, α -CH_3). MS (100 eV, 160°C): m/e (%) = 434 (65, M^+), 399 (44), 363 (64), 328 (72), 292 (49), 246 (75), 245 (29), 165 (100), 139 (89), 114 (32), 113 (46), 87 (11), 63 (14).

2-Methylnaphtho(1.8-bc) selenophen-3-(2', 3', 4',5'-tetracyanocyclopentadienyliden) (26b)

Aus 0.5 g (2 mmol) *(16)* und 0.1 g (2 mmol) 2,3,4,5-Tetracyanocyclopentadien in 10 ml Acetanhydrid analog *(26a)*. Violette Kristalle; Schmp. 132°C; Ausb. 0.2 g (24%).

$C_{21}H_8N_4Se$ (395.3) Ber. C 63.81 H 2.04 N 14.18
Gef. C 63.25 H 1.88 N 14.38

IR (KBr): 2200 (C≡N), 1570, 1525 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.71 (d, H^4), 7.14 - 8.02 (m, H^5, H^6, H^7, H^8), 2.90 (s, α - CH_3). MS (100 eV, 270°C): m/e (%) = 396 (82, M^+), 370 (69), 344 (68), 318 (52), 292 (54), 246 (86), 245 (27), 165 (86), 139 (100), 114 (27), 113 (46), 87 (6), 63 (7).

2-Methylnaphtho(1.8-bc)selenophen-3(2', 3', 4', 5'-tetramethoxy-carbonyl-cyclopentadienyliden (26 c)

Zu einer Lösung von 0.5 g (2 mmol) *(16)* in 10 ml Acetonitril werden 0.5 g (2 mmol), 2,3,4,5-Tetramethoxy-carbonyl-cyclopentadien[17] gegeben. Nach 6 stdgem Erhitzen unter Rückfluß wird der entstandene Niederschlag säulenchromatographisch gereinigt - Kieselgel 60 «Merck», Benzol als Elutionsmittel. Schwarzglänzende Kristalle (aus Methylenchlorid); Schmp. 217°C; Ausb. 0.16 g (15%).

$C_{28}H_{20}O_8$ Se (527.4) Ber. C 56.94 H 3.82
Gef. C 56.38 H 3.50

IR (KBr): 1720 (C=0), 1570, 1535 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 8.12 (d, H^4), 7.25 - 8.28 (m, H^5, H^6, H^7, H^8), 2.69 (s, α-CH_3). MS (100 eV, 254°C): m/e (%) = 528 (78, M^+), 514 (68), 500 (54), 472

(72), 469 (59), 427 (68), 410 (81), 382 (74), 351 (60), 337 (48), 292 (53), 246 (87), 245 (14), 165 (94), 139 (100), 114 (34), 113 (79), 87 (16), 63 (20).

2-Methylnaphtho(1.8-bc) selenophen-3- (2', 3', 4', 5'-tetraphenylcyclopentadienyliden) (26 d)

Zu einer Lösung von 1.24 g (5 mmol) *(16)* und 1.98 g (5 mmol) 2,3,4,5-Tetraphenyl-1-diazo-cyclopentadien in 15 ml Xylol/ Nitrobenzol wird eine katalytische Menge $AlCl_3$ gegeben und 6 h unter Rückfluß zum Sieden erhitzt; der entstandene Niederschlag wird säulenchromatographisch - Kieselgel 60 «Merck» - mit Benzol/ Tetrachlorkohlenstoff (1:1) als Elutions-mittel gereinigt. Schwarze glänzende Kristalle (aus n-Hexan/ Benzol); Schmp. 208°C; Ausb. 0.21 g (7%).

$C_{41}H_{28}Se$ (599.6) Ber. C 82.13 H 4.71 Gef. C 82.67 H 4.65. IR (KBr): 1570, 1530 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.54 (d, H^4), 7.32 - 8.28 (m, H^5, H^6, H^7, H^8), 2.64 (α -CH_3). MS (100 eV, 150°C): m/e (%) = 600 (72, M^+), 523 (67), 446 (70), 369 (56), 292 (53), 246 (81), 245 (23), 165 (89), 139 (100), 114 (34), 113 (52), 87 (17), 63 (14).

2-Methylnaphtho (1.8-b-c) selenophen-5-diphenyliden (27a)

Aus 0.5 g (2 mmol) *(13)* und 0.4 g (2 mmol) Diphenylketen in 10 ml wassefreiem Benzol analog *(24a)*. Gelbliche Kristalle; Schmp. 161°C; Ausb. 0.71 g (89%).

$C_{25}H_{18}Se$ (397.1) Ber. C 75.62 H 4.57 Gef. C 75.10 H 4.84 – IR (KBr): 1590, 1550 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.23 – 8.03 (m, H^3, H^7, H^8), 6.92 (d, H^4), 7.01 (d, H^6), 2.53 (s, α-CH_3). MS (100 eV, 160°C): m/e (%) = 398 (89, M^+), 322 (73), 246 (34), 245 (86), 165 (100), 139 (71), 114 (22), 113 (34), 87 (13), 63 (11).

2-Methylnaphtho (1.8-bc) selenophen-5-(tert. -butyl-cyanyliden) (27b)

Aus 0.5 g (2 mmol) *(13)* und 0.24 g (2 mmol) tert. -Butyl-cyanoketen in 10 ml wasserfreiem Benzol analog *(24 b);* es liegt ein Gemisch zweier Isomerer vor. Schmp. 156°C; Ausb. 0.52 g (80%)

$C_{18}H_{17}N$ Se (326.3) Ber. C 66.23 H 5.25 N 4.29
Gef. C 65.87 H 5.22 N 4.39

IR (KBr): 2120 (C≡N), 1585 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.53 - 8.92 (m, H^3, H^7, H^8), 6.79 (d, H^4 von Isomer α), 6.58 (d, H^4 von Isomer β), 2.58 (s, α -CH_3), 7.59 (d, H^6 von Isomer α), 6.58 (d, H^6 von Isomer β). MS (100 eV, 80°C): m/e (%) = 327 (82, M^+), 271 (53), 246 (30), 245 (78), 165 (100), 139 (64), 114 (20), 113 (25), 87 (13), 63 (9).

2-Methylnaphtho (1.8-bc) selenophen-5-dicyanomethylen (27c)

Aus 0.5 g (2 mmol) *(13)* und 0.067 g (2 mmol) Malonsäuredinitril in 10 ml Essigsäureanhydrid analog *(23)*. Rote Kristalle; Schmp. 297° C; Ausb. 0.095 g (16%).

$C_{15}H_8N_2$ Se (295.2) Ber. C 61.03 H 2.73 N 9.49
Gef. C 60.89 H 2.53 N 9.19

IR (KBr): 2220 (C≡N), 1570 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.92 - 8.52 (m, H^3, H^7, H^8), 7.98 (d, H^4), 8.09 (d, H^6), 2.79 (s, α-CH_3). MS (100 eV, 160°C): m/e (%) = 296 (59, M^+), 271 (38), 246 (64), 245 (23), 165 (100), 139 (86), 114 (19), 113 (37), 87 (9), 63 (11).

2-Methylnaphtho (1.8-bc)selenophen-5 (pyrimidin-2', 6'- dion-4'-thion-5'-yliden) (28)

Aus 0.5 g (2 mmol) *(13)* und 0.34 g (2 mmol) 2-Thiobarbitursäure in einer Mischung aus 8 ml Acetanhydrid und 4 ml Eisessig analog *(22)*. Grüne Nadeln; Schmp. 326°C; Ausb. 0.5 g (64%).

$C_{16}H_{10}N_2O_2S$ Se (373.3) Ber. C 51.48 H 2.70 N 7.50
Gef. C 51.85 H 2.47 N 7.19

IR (KBr): 1695, 1655 cm^{-1} (C=O). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.14 - 8.35 (m, H^3, H^7, H^8), 8.02 (d, H^4), 8.14 (d, H^6), 2.61 (s, α -CH_3). MS (100 eV, 145°C): m/e (%) = 374 (64, M^+), 314 (37), 287 (29), 273 (47), 246 (69), 245 (23), 165 (100), 139 (94), 114 (27), 113 (56), 87 (11), 63 (15).

2-Methylnaphtho (1.8-bc) selenophen-5-fluorenyliden (29)

Aus 0.5 g (2 mmol) *(13)* in 10 ml wasserfreiem Benzol und 0.4 g (2 mmol) Fluorenketen analog *(25)*. Blaue Kristalle; Schmp. 145°C; Ausb. 0.96 g (24%).

$C_{25}H_{16}Se$ (395.3) Ber. C 75.95 H 4.08
Gef. C 75.76 H 87

IR (KBr): 1560, 1510 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.21 - 8.25 (m, H^3, H^7, H^8), 7.30 (d, H^4), 7.49 (d, H^6), 2.92 (s, α -CH_3). MS (100 eV, 140°C): m/e (%) = 396 (7, M^+), 246 (78), 245 (34), 165 (11), 139 (69), 114 (16), 113 (37), 87 (23), 63 (12).

2-Methylnaphtho (1.8-bc) selenophen-5-(2', 3', 4', 5'- tetrachlorocyclopentadienyliden) (30 a)

Aus 0.5 g (2 mmol) *(13)* in 10 ml Acetanhydrid und 0.2 g (2 mmol) 2, 3, 4, 5 - Tetrachlorocyclopentadien analog *(26 a)*. Blaue Kristalle; Schmp. 159°C; Ausb. 0.2 g (23%).

$C_{17}H_8Cl_4Se$ (433.0) Ber. C 47.15 H 1.86
Gef. C 46.93 H 2.02

IR (KBr): 1580, 1530 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.12 - 8.20 (m, H^3, H^7, H^8), 8.09 (d, H^4), 8.69 (d, H^6), 2.82 (s, α-CH_3). MS (100 eV, 136°C): m/e (%) = 434 (65, M^+), 399 (44), 363 (64), 428 (72), 292 (49), 246 (75), 245 (29), 165 (100), 139 (89), 114 (32), 113 (47), 87 (13), 63 (18).

2-Methylnaphtho(1.8-bc) selenophen-5-(2', 3', 4', 5' tetracyanocyclopentadienyliden) (30 b)

Aus 0.5 g (2 mmol) *(13)* in 10 ml Acetanhydrid und 0.1 g (2 mmol) 2, 3,

4, 5- Tetracyano-cyclopentadien analog *(26 a)*. Blaue Kristalle. Schmp. 160°C; Ausb. 0.15 g (19%).

$C_{21}H_8N_4Se$ (395.3) Ber. C 63.81 H 2.04 N 14.18
Gef. C 63.59 H 1.96 N 14.49

IR (KBr): 2200 (C≡N), 1575, 1520 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.23 - 8.28 (m, H^3, H^7, H^8), 8.59 (d, H^4), 8.74 (d, H^6), 2.84 (s, α-CH_3). MS (100 eV, 264°C): m/e (%) = 396 (76), 370 (81), 344 (72), 318 (65), 292 (65), 246 (79), 245 (28), 165 (100), 139 (85), 114 (46), 113 (46), 87 (23), 63 (24).

2-Methyl-naphtho (1.8-bc) selenophen-5- (2', 3', 4', 5' -tetramethoxycarbonyl-cyclopentadienyliden) (30 c)

Aus 0.5 g (2 mmol) *(13)* in 10 ml Acetonitril und 0.47 g (2 mmol) 2,3,4,5, *-Tetramethoxy-carbonyl-cyclopentadien*[17] analog (26 c). Blauschwarze Kristalle (aus Methylenchlorid); Schmp. 198°C; Ausb. 0.3 g (27%).

$C_{25}H_{20}O_8$ Se (527.4) Ber. C 56.94 H 3.82
Gef. C 56.70 H 4.18

IR (KBr): 1720 (C=O), 1570, 1530 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.34 - 8.51 (m, H^3, H^7, H^8), 8.25 (d, H^6), 7.95 (d, H^4), 2.63 (s, α - CH_3). MS (100 eV, 250°C): m/e (%) = 528 (86, M^+), 514 (67), 472 (52), 469 (68), 427 (59), 410 (46), 382 (58), 351 (74), 337 (75), 292 (46), 246 (59), 245 (24), 165 (93), 139 (100), 114 (16), 113 (49), 87 (19), 63 (17).

2-Methylnaphtho (1.8-bc) selenophen-5- (2', 3', 4', 5' -tetraphenyl-cyclopentadienyliden) (30 d)

Aus 1.24 g (5 mmol) *(13)* in 15 ml Xylol/Nitrobenzol und 1.98 g (5 mmol) 2,3,4,5 -Tetraphenyl-diazo-cyclopentadien analog *(26 d)*. Schwarze Kristalle; Schmp. 276°C; Ausb. 0.12 g (4%).

$C_{41}H_{28}Se$ (599.6) Ber. C 82.13 H 4.71
Gef. C 82.50 H 4.42

IR (KBr): 1575, 1525 cm^{-1} (C=C). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.47 - 8.46 (m, H^3, H^7, H^8), 7.95 (d, H^4), 7.52 (d, H^6), 2.56 (s, α- CH_3). MS (100 eV, 150°C): m/e (%) = 600 (65, M^+), 523 (72), 446 (64), 369 (57), 292 (43), 246 (78), 245 (34), 165 (100), 139 (89), 114 (23), 113 (78), 87 (19), 63 (9).

5-Ethoxy-5-methylen-naphtho (1.8-bc) selenophen (31)

Zu einer Lösung von etwa 0.23 g (2 mmol) *(14)* in 5 ml Acetonitril - wasserfrei - werden 0.22 g (4 mmol) Anilin getropft und anschließend noch einige Tropfen Ethyl-diisopropylamin. Nach Entfernen des Lösungsmittels wird der Rückstand säulenchromatographisch gereinigt - über Kieselgel 60 «Merck», mit Benzol als Elutionsmittel. Gelbe Kristalle (aus Ethanol). Schmp. 116°C; Ausb. 0.13 g (24%).

$C_{14}H_{12}O$ Se (275.2) Ber. C 61.10 H 4.40
Gef. C 60.66 H 4.35

^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 7.59 - 8.63 (m, H^3, H^6, H^7, H^8), 6.60 (s, H^4), 4.16 (q, -O-CH_2), 1,46 (t, -CH_3), 6.92 (d, α – CH_2). MS (100 eV, 90°C): m/e (%) = 276 (100, M^+), 250 (40), 218 (79), 174 (90).

3-Ethoxy-2-methylen-naphtho (1.8-bc) selenophen (32)

Aus etwa 0.24 g (2 mmol) *(17)*, 0.22 g (4 mmol) Anilin und einigen Tropfen Ethyldiisopropylamin analog *(31)*. Orangefarbene Kristalle; Schmp. 134°C; Ausb. 0.11 g (20%).

$C_{14}H_{12}O$ Se (275.1) Ber. C 61.10 H 4.40
Gef. C 61.47 H 4.31

IR (KBr): 1620 cm^{-1} (C=CH_2). ^{1}H-NMR (90 MHz, $CDCl_3$): δ (ppm) = 6.51 (s, H^4), 7.83 - 8.64 (m, H^5, H^6, H^7, H^8), 6.81 (d, α – CH_2), 4.06 (q, –O–CH_2-), 1.22 (t, -CH_3). MS (100 eV, 110°C): m/e (%) = 276 (100, M^+), 250 (46), 218 (73), 174 (89).

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Summary

Heterocyclic 12-π- and 14-π- systems, 38:
Syntheses and Reactivities of new Selenapseudophenalenone-derivatives

The reaction of diazotated anthranilic acid with Na_2Se_2 yielded «diselenosalicylic acid» *(2)*. Reduction of *(2)* and subsequent esterification yielded the dimethylester of the o-carboxyphenyl-selenoacetic acid *(4)*, which by Dieckmann- condensation gave the α-ketocarboxylic acid-ester *(5)*. From methylation of *(5)* with methyliodide followed by hydrolysis and decarboxylation we obtained *(7)*, which after reduction to the alcohol *(8)* and elimination of water yielded 2-methylbenzo(b) selenophene *(9)*. This compound *(9)* reacted with α-chloro-dimethyl-ether to the chloro-methyl-derivative *(10)*, which was converted according to known methods to the propionic acid *(11)*. By cyclisation of *(11)* to the ketone *(12)* and oxidation we finally obtained the selenapseudo-phenalenone-5, which by alkylation with «Meerwein-salt» yielded the selenapseudophenaleniumtetra-fluoroborate *(14)*. Reaction of *(9)* with cinnamoylchloride in the presence of $AlCl_3$ yielded the isomeric ketone *(16)*, and subsequently the kation *(17)* by alkylation. In addition, from *(13)* we obtained by known methods the imino-selena-pseudophenalenone-derivatives *(18 a - 18 c)* and the azine *(19)*. Similarly from *(16)* we prepared the imino-derivatives *(20 a - 20 c)* and *(21)*, and from the reaction of *(16)* with C-nucleophiles the selenapseudophenafulvenes *(22, 23)* and *(24 a - 24 b)*. Reaction of *16* and *13* with the corresponding N-nucleophiles gave the selenapentapseudophenafulvalenes *(25, 26 a - 26 d)* and *(29), (30 a - 30d)* respectively. The reactions of the selenapseudophenalenone-5 *(13)* with C-nucleophiles yielded the selena-pseudophenafulvenes *(27 a - 27 c)* and *(28)*. By elimination of a proton from the cations *(14)* and *(17)* we isolated the compounds *(31)* and *(32)*.

Περίληψη

Ἑτεροκυκλικὰ 12-π- καὶ 14-π- συστήματα
Σύνθεση καὶ ἰδιότητες νέων σεληνα-ψευδο-φαιναλενονικῶν παραγώγων

Ἀπὸ τὴν ἀντίδραση διαζωτωθέντος ἀνθρανιλικοῦ ὀξέος *(1)* μὲ Na_2Se_2 λαμβάνεται ἀρχικὰ τό «δισεληνο-σαλικυλικὸ ὀξύ» *(2)*, μὲ ἀναγωγὴ τοῦ ὁποίου παίρνουμε τὸ ο-καρβοξυφαινυλοσεληνοξικὸ ὀξύ *(3)*, ποὺ περαιτέρω μετατρέπεται στὸ διμεθυλεστέρα του *(4)* καὶ στὴ συνέχεια στὸν α-κετοκαρβοξυλικὸ ἐστέρα *(5)* μὲ συμπύκνωση κατὰ Dieckmann. Ἀπὸ τὸ *(5)* λαμβάνεται ἡ ἕνωση *(6)* μετὰ ἀπὸ μεθυλίωση μὲ μεθυλιωδίδιο καὶ τελικὰ μὲ σαπωνοποίηση καὶ ἀποκαρβοξυλίωση τὸ 2,3-διυδρο-2-μεθυλο-3-οξο-βενζο(b) σεληνοφαίνιο *(7)*. Ἀναγωγὴ τοῦ *(7)* δίνει τὴν ἀλκοόλη *(8)*, καὶ μὲ ἀπόσπαση ὕδατος λαμβάνεται τὸ 2-μεθυλο-βενζο(b)σεληνοφαίνιο *(9)*. Ἀπὸ τὸ *(9)* μὲ ἐπίδραση α-χλωρο-διμεθυλαιθέρα λαμβάνεται τὸ 3-χλωρομεθυλο-παράγωγο *(10)* καὶ κατόπι μὲ γνωστὴ μέθοδο τὸ 3 (2-μεθυλοβενζο(b)σεληνιενυλο)προπιονικὸ ὀξύ *(11)*. Κυκλοποίηση τοῦ *(11)* δίνει τὴν κετόνη *(12)*, καὶ μὲ ὀξείδωσή της λαμβάνεται ἡ σεληνα-

ψευδοφαιναλενόνη-5 *(13)*. Αὐτὴ ἀλκυλιώνεται εὔκολα μὲ ἅλας Meerwein (τετραφθοροβορικὸ τριαιθυλοξώνιο) πρὸς τὸ τετραφθοροβορικὸ ἅλας τοῦ 2-μεθυλο-5-αιθοξυ-ναφθο(1.8-bc) σεληνονίου *(14)*.

Ἀπὸ τὴν ἔνωση *(9)* καὶ χλωρίδιο κινναμωμικοῦ ὀξέος παρουσία τριχλωριούχου ἀργιλλίου λαμβάνεται ἡ ἰσομερὴς κετόνη σεληνα-ψευδοφαιναλενόνη-3 *(16)*, μὲ ἀλκυλίωση τῆς ὁποίας συντίθεται τὸ τετραφθοροβορικὸ ἅλας τοῦ 2-μεθυλο-3-αιθοξυ-ναφθο(1.8-bc)σεληνονίου *(17)*.

Ἀπὸ τὸ *(13)* λαμβάνονται τὰ ἰμινο-σεληνα-ψευδοφαιναλενονικὰ παράγωγα *(18a-18c)* καὶ ἡ ἀζίνη *(19)*. Ἀπὸ τὴν κετόνη *(16)* μὲ ἀνάλογο τρόπο παίρνουμε τὰ ἰμινο-παράγωγα *(20a-20c)*. Ἀπὸ τὸ *(16)* μὲ ἑνώσεις μὲ ἐνεργὸ μεθυλενικὴ ὁμάδα λαμβάνονται τὰ σεληνα-ψευδο-φαιναφουλβαλένια *(22, 23, 24a-24b)*.

Ἐπίσης τὸ *(13)* ἀντιδρᾶ πρὸς τὰ σεληνα-πεντα-ψευδο-φαιναφουλβαλένια *(29, 30a-30d)*, καί *(16)* ἀνάλογα πρὸς τά *(25, 26a-26d)*. Ἡ ἀντίδραση τέλος τῆς σεληνα-ψευδο-φαιναλενόνης-5 *(13)* μὲ ἑνώσεις μὲ ἐνεργὸ μεθυλενικὴ ὁμάδα ἔδωσε τὰ σεληνα-ψευδο-φαιναφουλβένια *(27a-27c, 28)*.

Ἡ ἀπόσπαση πρωτονίου ἀπὸ τὰ κατιόντα *(14)* καί *(17)* ὁδήγησε στὶς ἑνώσεις 5-αἰθοξυ-2-μεθυλενο-ναφθο (1.8-bc)σεληνοφαίνιο *(31)* καί *(32)*.

Μελετήθηκαν τὰ φασματοκοπικὰ δεδομένα IR, NMR καὶ μαζῶν ὅλων τῶν νέων ἑνώσεων, ἀπὸ τὰ ὁποῖα συνάγεται ὅτι στὴ βασικὴ ἠλεκτρονικὴ κατάσταση τῶν ἑνώσεων *(13)* καί *(16)* συμμετέχει κατὰ μεγάλο ποσοστὸ ἡ διπολικὴ ὁριακὴ δομή (πβ. *14* καὶ *17*).

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MASS SPECTRA STUDY OF ORGANOSILANES: A NOVEL 1,4-HYDROXYL MIGRATION FROM CARBON TO SILICON IN HYDROXY-SILYL-ALKENES

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Summary

The mass spectra of some silyl-alkenes and hydroxy-silyl-alkenes are studied. Fragmentation patterns are given and possible mechanisms are discussed. In the case of the hydroxy-silyl-alkenes a novel 1,4-hydroxyl migration from carbon to silicon was observed and its mechanism is discussed. In this type of rearrangement a γ hydroxyl group is transfered to an unsaturated siliconium center accompanied by the expulsion of a neutral substituted cyclopropene derivative.

Key words: mass spectra, silyl-alkenes, hydroxy-silyl-alkenes, 1,4-hydroxyl migration, cyclopropene derivative.

Introduction

A large number of various types of organosilanes have been studied mass spectrometrically and some novel intramolecular migrations of siliconium ions appear in the fragmentation processes[1-6]. These intramolecular rearrangements involve the silyl center and were observed in a number of silyl derivatives of alcohols[7,8], carboxylic acids[9] and other functional groups[10,11]. The characteristic feature of these rearrangements is the migration of the silyl group from the γ carbon of the alkyl chain to the positively charged oxygen of the functional group with simultaneous elimination of ethylene. Another important migration of a phenyl group to the silyl center of a siliconium ion was also observed in the fragmentation process of 1-phenyl-2-trimethylsilyl-ethane and trimethylsilylbenzyl ethers[3]. In the present paper we describe the general fragmentation patterns of silyl-alkenes and some hydroxy-silyl-alkenes. In the last case a novel 1,4-hydroxyl migration from carbon to silicon was observed, which is also discussed.

Results and discussion

In Table I are shown the most relevant mass spectral peaks of some silyl-alkenes under study. The molecular ions in the mass spectra of trans-1-triethylsilyl-1-alkenes exhibit a low abundance in accordance with the mass spectra of other tetraalkylsilanes[6]. The low abundance of the molecular ions could be explained on the basis of their instability, for they are odd-electron

ions that can eliminate easily a radical to form the stable even-electron siliconium $|R_3Si|^+$ or $|R'R_2Si|^+$ (where R= ethyl group, Et, and R'= alkenyl group) ions. The particularly stable even-electron siliconium ions exhibit, as one would expect, the highest abundance and their peaks are the base peaks in the mass spectra of the trans-1-triethylsilyl-1-alkenes.

TABLE I. *Most relevant mass spectral peaks of some silyl-alkenes.*

	m/e (R.I.)*	
trans-$CH_3(CH_2)_2CH{=}CHSi(C_2H_5)_3$	:	184 (13), 155 (100), 150 (9), 149 (9), 135 (12), 127 (83), 115 (5), 110 (12), 99 (29), 87 (15), 71 (9), 59 (29), 57 (10), 55 (6), 44 (18), 43 (10).
trans-$CH_3(CH_2)_3CH{=}CHSi(C_2H_5)_3$	:	198 (27), 169 (100), 141 (92), 115 (traces), 113 (41), 99 (7), 87 (25), 85 (20), 83 (11), 81 (5), 73 (6), 71 (10), 69 (6), 59 (41), 57 (14), 55 (7), 45 (7), 43 (11).
trans-$CH_3(CH_2)_4CH{=}CHSi(C_2H_5)_3$	:	212 (9), 183 (100), 155 (52), 127 (11), 115 (6), 113 (5), 99 (8), 87 (17), 85 (9), 83 (6), 73 (5), 71 (6), 59 (25), 57 (8), 43 (6).
trans-$(CH_2)_4CHCH{=}CHSi(C_2H_5)_3$	:	210 (22), 181 (100), 153 (75), 125 (29), 115 (15), 113 (9), 97 (16), 95 (9), 87 (35), 85 (15), 83 (10), 59 (49), 57 (13), 55 (7), 45 (6), 43 (7).
cis-$CH_3CH_2C(Si(C_2H_5)_3){=}CHCH_2CH_3$	:	198 (21), 169 (100), 141 (74), 115 (19), 113 (24), 111 (5), 99 (9), 87 (36), 85 (9), 83 (6), 73 (9), 71 (5), 59 (40), 45 (6), 43 (7).

*(R.I.)= Relative Intensity.

Two types of the siliconium ions could be formed from the parent ions after the elimination of the alkyl radical. The first type of the siliconium ion corresponds to the siliconium ion $|R'R_2Si|^+$ which is formed after the elimination of an ethyl radical from the triethylsilyl group and the second one $|R_3Si|^+$ from the elimination of an alkenyl radical.

The first type siliconium ions constitute the major peaks (base peaks) in the mass spectra of the trans-1-triethylsilyl-1-alkenes, while the second type appears in very low abundance. The easier detachment of the ethyl radical than the alkenyl radical may be due to the stronger Si-C bond between the alkenyl group and silicon than the ethyl group and silicon. In the first case the extra stabilization of the Si-C bond may be due to the delocalization of the π-electron density between carbon and silicon through the π-back bonding effect.

In figure 1 is shown the general fragmentation pattern of a representative trans-1-triethylsilyl-1-alkene, namely the trans-1-triethylsilyl-1-hexene. The fragmentation of the other trans-1-triethylsilyl-1-alkenes under study occur by the same pathways. Most of the fragmentation processes were metastable supported.

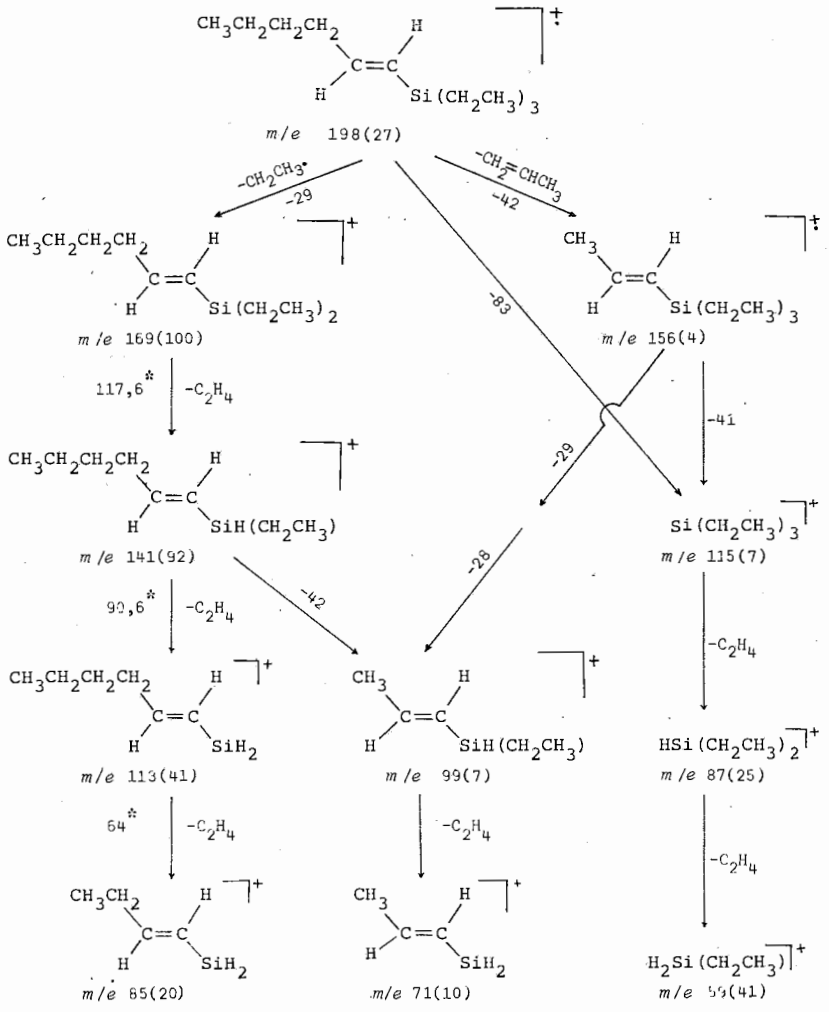


FIG. 1. *Possible fragmentation pattern of the trans-1-triethylsilyl-1-hexene (Numbers in parentheses are the relative intensities of the corresponding peaks).*

The salient feature of the general fragmentation pattern is the stepwise elimination of ethylene either from the first type or from the second type siliconium ions. Stepwise elimination of ethylene was also observed in the case of other tetraalkylsilanes studied mass spectrometrically[6,12].

Examination of the mass spectra of the compounds trans-1-triethylsilyl-2-cyclopentyl-ethylene, trans- $(CH_2)_4CHCH{=}CHSiEt_3$, and trans-3-hydroxy-3-methyl-1-triethysilyl-but-1-ene, trans-$Me_2C(OH)CH{=}CHSiEt_3$, reveals the fact that the ethyl radical is eliminated from the triethylsilyl group and not from the alkenyl group. In these two compounds there is no possibility for elimination of the ethyl group from the alkenyl group.

The stepwise elimination of ethylene, which is metastable supported, is also characteristic in the fragmentation processes of the compounds trans-$Me_2C(OH)CH{=}CHSiEt_3$, trans-$MeEtC(OH)CH{=}CHSiEt_3$, cis-$Me_2C(OH)C(SiEt_3) = CHC(OH)Me_2$ and cis-$CH_3CH_2C(SiEt_3) = CHCH_2CH_3$.

The mass spectrum of the compound trans-$Me_2C(OH)CH{=}CHSiEt_3$ together with the proposed fragmentation pathways are shown in figure 2. Support for the fragmentation pathways comes from the presence of metastable peaks. Analogous fragmentation processes appear also in the case of the compound trans-$MeEtC(OH)CH{=}CHSiEt_3$.

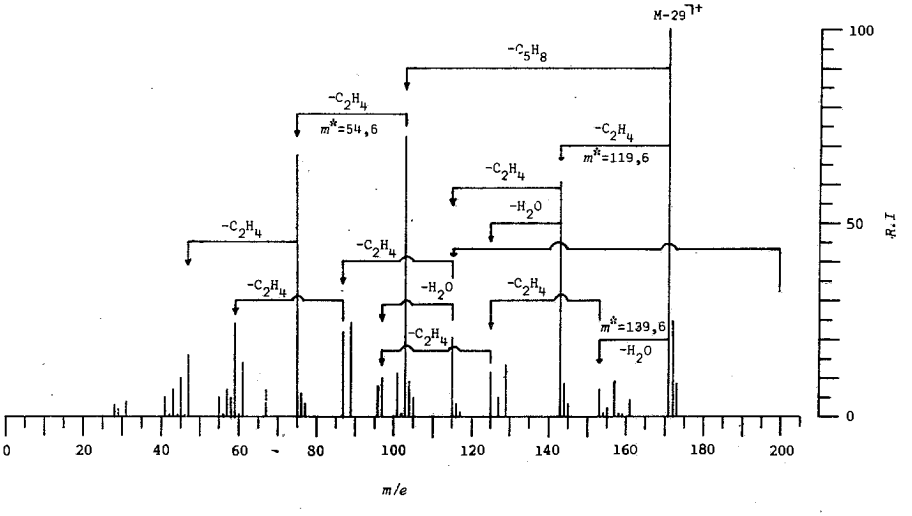
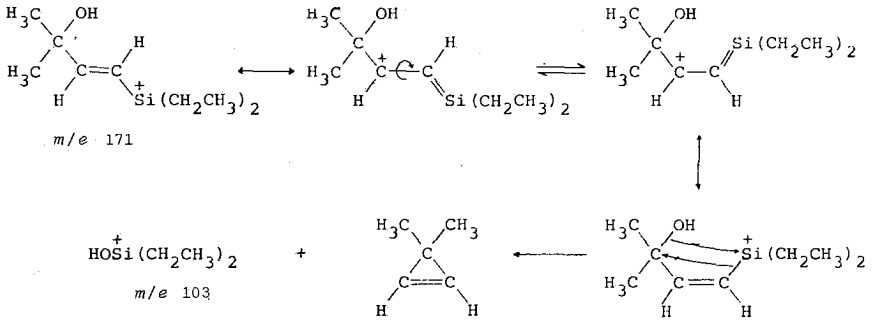


FIG. 2. *Mass spectrum of the trans-Me_2C (OH) CH= CHSiEt$_3$, showing as well as the proposed fragmentation pathways.*

The feature to be noted in the mass spectra of the hydroxy-silyl-alkenes is the existense of a peak at m/e 103, having very high intensity. No such peak appears in the mass spectra of the trans-1-triethylsilyl-1-alkenes. This peak is due to the ionic fragment $|(CH_3CH_2)_2SiOH|^+$ which results from the fragmenta-

tion of the first type siliconium ions discussed previously. The formation of the ion $|(CH_3CH_2)_2SiOH|^+$ can be explained according to the following proposed mechanism:

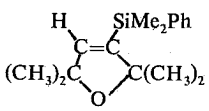
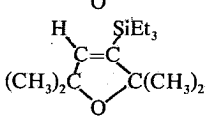


The proposed mechanism involves the isomerisation of the trans-siliconium ion to the isomer followed by an intramolecular transfer of the γ hydroxyl group to the unsaturated siliconium center, which has as a result the expulsion of a neutral cyclopropene derivative and the formation of the ion $|(CH_3CH_2)_2SiOH|^+$. This intramolecular rearrangement is analogous to the McLafferty rearrangement of methyl esters[12], as well as to other silyl McLafferty type rearrangements observed in some organosilicon compounds[1,3,5,6]. The only difference in this type of rearrangement is the formation of a cyclopropene derivative rather than ethylene.

The formation of the $|R_2SiOH|^+$ ion is also characteristic in the mass spectra of the compounds cis-2,5-dihydroxy-2,5- dimethyl-3-triethylsilyl-hex-3-ene, cis-$HOMe_2CC(SiEt_3)$=$CHCMe_2OH$, and cis-2,5-dihydroxy-2,5- dimethyl-3-dimethylphenylsilyl-hex-3-ene, cis-$HOMe_2CC(SiMe_2Ph)$ = $CHCMe_2OH$. In Table II are given the most relevant mass spectral peaks of some hydroxy-silyl-alkenes under study. Also in Table II are shown the mass spectral peaks of the compounds 2, 2, 5, 5,-tetramethyl-3-triethylsilyl- dihydrofurane and 2, 2, 5, 5-tetramethyl-3- dimethylphenylsilyl- dihydrofurane. The last two compounds are the dehydration products of the two dihydroxy- silyl-alkenes under study.

The base peak in the mass spectra of the dihydroxy- silyl-alkenes corresponds to the siliconium ions $|Et_3Si|^+$ (m/e 115) and $|Me_2PhSi|^+$ (m/e 135). These peaks are also the base peaks in the mass spectra of the dehydration products of the dihydroxy-silyl-alkenes studied. The absense of the molecular ion in the mass spectra of the dihydroxy-silyl-alkenes studied, is indicative of their easy dehydration, because of their cis-structure. The dehydration products are silyl derivatives of the dehydrofurane. The fragment ions with the higher m/e values correspond to the $|M-15|^+$ and $|M-18|^+$ ions. The easy formation of

TABLE II. *Most characteristic mass spectral peaks of some hydroxy-silyl-alkenes and silyl derivatives of dihydrofurane.*

trans-$Me_2C(OH)CH{=}CHSiEt_3$	m/e(R.I.)*	: 171 (100), 161 (4), 157 (9), 153 (7), 143 (60), 129 (13), 125 (12), 115 (21), 103 (72), 97 (10), 89 (24), 87 (22), 75 (67), 67 (7), 61 (14), 59 (24), 47 (16), 45 (10), 43 (8).
trans-$MeEtC(OH)CH{=}CHSiEt_3$		: 196 (3), 185 (88), 139 (16), 129 (23), 115 (40), 103 (100), 87 (43), 75 (89), 59 (35), 47 (20), 45 (12), 43 (14).
cis-$HOMe_2CC(SiMe_2Ph){=}CHCMe_2OH$		: 263 (34), 260 (43), 245 (97), 202 (52), 187 (31), 183 (47), 143 (61), 137 (99), 135 (100), 109 (58), 107 (23), 105 (19), 91 (26), 83 (19), 77 (20), 75 (73), 68 (52), 67 (41), 59 (70), 45 (17), 43 (47).
cis-$HOMe_2CC(SiEt_3){=}CHCMe_2OH$		: 243 (3), 240 (5), 225 (52), 211 (26), 182 (8), 154 (27), 153 (21), 115 (100), 109 (45), 103 (66), 87 (74), 75 (54), 67 (24), 59 (41), 47 (16), 43 (15).
		: 260 (11), 245 (100), 202 (4), 187 (7), 183 (11), 135 (99), 107 : (37), 91 (19), 75 (31), 59 (11), 43 (32)
		: 240 (traces), 225 (94), 211 (9), 196 (2), 183 (4), 167 (3), 153 (2), 139 (3), 125 (5), 115 (100), 87 (72), 75 (14), 59 (22), 43 (7).

*(R.I.) = Relative Intensity.

the dehydrofurane derivatives explains also the lower abundance of the ions $|Et_2SiOH|^+$ and $|MePhSiOH|^+$ or $|Me_2SiOH|^+$ in the mass spectra of the dihydroxy-silyl-alkenes than that in the hydroxy-silyl-alkenes. Of course there are no peaks corresponding to the above ions in the mass spectra of the silyl derivatives of the dehydrofurane studied.

Experimental

The compounds studied were prepared by hydrosilylation of the corresponding alkynes using trans-di-μ-hydrido-bis (tricyclohexylphosphine) bis (dimethylbenzylsilyl) diplatinum, $|(Cy_3P)\ (Me_2BzSi)\ (\mu\text{-}H)\ Pt|_2$ as catalyst. The catalyst was prepared as described in the literature[13].

The description of hydrosilylation experiments will be limited to only one representative case, as the full results on the hydrosilylation of alkynes

catalysed by trans-di-μ-hydrido-bis(tricyclohexylphosphine)bis(silyl)diplatinum complexes will be published elsewhere[14].

A glass tube (c.a 80 cm^3) fitted with a Westef high-vacuum greaselles stopcock and standard tapered joint was charged with 3.2 cm^3 (20.0 mmol) freshly distilled Et_3SiH, 1.0cm^3 (10.0 mmol) freshly distilled 2-methyl-butyn-3-ol-2 and 2.0 mg of the catalyst $|(Cy_3P)\ (Me_2BzSi)\ (\mu\text{-}H)\ Pt|_2$, under argon atmosphere. After 6 hrs at 60°C a ^{1}H-NMR spectrum revealed complete consumption of the alkyne. ^{1}H-NMR, ^{13}C-NMR and G.L.C. studies showed that the distilled product (1,9 g, 95%) consisted only of trans- $Me_2C(OH)\ CH{=}CHSiEt_3$. Analogous hydrosilylation reactions of appropriate alkynes were used for the preparation of the other silyl-alkenes and hydroxy-silyl-alkenes under study. Analytical data for the new compounds were given elsewhere[14].

The dihydrofurane derivatives were prepared according to the following method.

A Schlenk tube was charged, under nitrogen atmosphere, with 1.39 g (5.0 mmol) of 2,5-dimethyl-2,5-dihydroxy-3-dimethylphenylsilyl-3-hexene, 1.36 g (10.0 mmol) $KHSO_4$ and 0.4 g of hydroquinone (as antioxidant). The mixture was heated at c.a. 100°C for 1 hr. After this period the mixture was cooled at room temperature and then was exctracted with ~15 cm^3 of n-hexane. The hexane solution was filtered and the filtrate by fractional distillation gave 1.2 g (~4.6 mmol) of 2,2,5,5-tetramethyl-3- dimethylphenylsilyl- dihydrofurane (colorless liquid, b.p. 275°C at 760 Torr). Yield ≃92%.

Calcd for $C_{16}H_{24}OSi$	C = 73.79%	H = 9.29%
Found	C = 73.71%	H = 9.32%

An analogous procedure was followed for the preparation of the 2,2,5,5-tetramethyl-3-triethylsilyl-dihydrofurane (colorless liquid, b.p. 245-246°C at 760 Torr, lit.[15] 74-77°C at 2 Torr). Yield = 98%.

Calcd for $C_{14}H_{28}OSi$	C = 69.93%	H = 11.74%
Found	C = 69.87%	H = 11.88%

The mass spectra were performed on a RMU-6L Hitachi-Perkin Elmer single focusing mass spectrometer. The operating conditions were, 70 eV electron energy, using the direct probe insertion of the samples at ~100°C. The used ion source is a T-2p model.

Περίληψη

Μελέτη τῶν φασμάτων μαζῶν ὀργανοπυριτικῶν ἐνώσεων. Μιὰ νέα 1,4-μετάθεση ὑδροξυλίου ἀπὸ ἄνθρακα σὲ πυρίτιο στὰ ὑδροξυ-σιλυκο-αλκένια.

Στὴν ἐργασία αὐτὴ μελετοῦνται τὰ φάσματα μαζῶν ὁρισμένων σιλυλο-αλκενίων καὶ ὑδροξυ-σιλυλο-αλκενίων. Δίνονται οἱ πιθανοὶ τρόποι διασπάσεως τῶν μοριακῶν ἰόντων καὶ προτείνονται πιθανοὶ μηχανισμοί. Οἱ περισσότεροι ἀπὸ τοὺς προτεινόμενους μηχανισμοὺς διασπάσεως ἐπιβεβαιώνονται ἀπὸ τὴν παρουσία μεταστάθῶν κορυφῶν.

Στὰ φάσματα μαζῶν τῶν σιλυλο-αλκενίων ἡ κορυφὴ ποὺ ἀντιστοιχεῖ στὸ μοριακὸ ἰὸν ἔχει πολὺ μικρὴ σχετικὴ ἔνταση, γεγονὸς ποὺ ἀποδίδεται στὴν ἀστάθεια τῶν μοριακῶν ἰόντων, ἀφοῦ αὐτὰ εἶναι ἰόντα περιττοῦ ἀριθμοῦ ἠλεκτρονίων, τὰ ὁποῖα ἀποβάλλουν εὔκολα μία ἀλυκο-ρίζα καὶ μεταπίπτουν στὰ ἀρτίου ἀριθμοῦ ἠλεκτρονίων σιλικωνιόντα, $|R'R_2Si|^+$ (ὅπου R′= ἀλκενύλιο καὶ R= ἀλκύλιο). Ἡ ρίζα ποὺ ἀποσπᾶται εὐκολότερα ἀντιστοιχεῖ σὲ ἀλκυλο-ρίζα καὶ ὄχι σὲ ἀλκενυλο-ρίζα. Τὰ σιλικωνιόντα $|R'R_2Si|^+$ δίνουν κορυφὲς ποὺ ἀποτελοῦν, κατὰ κανόνα, καὶ τὶς βασικὲς κορυφὲς στὰ φάσματα μαζῶν τῶν σιλυλο-αλκενίων.

Στὰ φάσματα μαζῶν τῶν ὑδροξυ-σιλυλο-αλκενίων ἰδιαίτερο ἐνδιαφέρον παρουσιάζει μία κορυφὴ ποὺ ἀπαντᾶ σὲ τιμὴ m/e 103. Ἡ κορυφὴ αὐτὴ ἀντιστοιχεῖ στὸ σιλικωνιὸν $|(C_2H_5)_2SiOH|^+$. Ὁ σχηματισμὸς τοῦ ἰόντος αὐτοῦ δικαιολογεῖται μὲ ἕνα εἶδος 1,4-μεταθέσεως ὑδροξυλίου, ποὺ λαμβάνει χώρα στὸ σιλικωνιὸν $|XR_2Si|^+$ (ὅπου X= ὑδροξυ-αλκενύλιο καὶ R= αἰθύλιο) καὶ γιὰ τὴν ὁποία προτείνεται πιθανὸς μηχανισμός. Σύμφωνα μὲ τὸ μηχανισμὸ αὐτὸ ἔχουμε ἀρχικὰ ἰσομερείωση τοῦ trans-σιλικωνιόντος στὸ cis ἰσομερές του, στὸ ὁποῖο γίνεται στὴ συνέχεια μετανάστευση τοῦ ὑδροξυλίου τῆς γ θέσεως στὸ ἀκόρεστο κέντρο τοῦ σιλικωνιόντος μὲ ταυτόχρονη ἀπόσπαση παραγώγου τοῦ κυκλοπροπενίου.

Στὰ φάσματα μαζῶν τῶν διυδροξυ-σιλυλο-αλκενίων ἀπαντᾶ ἐπίσης ἡ κορυφὴ m/e 103 ἀλλὰ μὲ μικρότερη ἔνταση. Ἐπίσης χαρακτηριστικὸ γνώρισμα τῶν φασμάτων μαζῶν τῶν διυδροξυ-σιλυκο-αλκενίων εἶναι ἡ ἀπουσία τοῦ μοριακοῦ ἰόντος γεγονὸς ποὺ ἀποδίδεται στὴν εὔκολη ἀπόσπαση ἑνὸς μορίου ὕδατος μὲ σχηματισμὸ σιλυλοπαραγώγων τοῦ διυδροφουρανίου.

Τέλος χαρακτηριστικὸ γνώρισμα τῶν φασμάτων μαζῶν τῶν μελετουμένων ἑνώσεων εἶναι ἡ σταδιακὴ ἀπόσπαση μορίων αἰθυλενίου ἀπὸ τὴ σιλυλο-ὁμάδα.

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THE THIOL SEQUENCES AND SUBUNIT STRUCTURE OF MUSCLE PHOSPHORYLASE

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Summary

Phosphorylase a and b (α-1, 4-glucan: orthophosphase glycosyltransferase, EC 2.4.1.1) have previously been shown to consist of four and two subnits, respectively, each having a molecular weight of 97,412. Our previous studies have indicated that of the nine sulfhydryl groups per monomer of the enzyme, two are alkylated rapidly without loss of enzymic activity and a further two are alkylated concomitantly with loss of activity and dissociation of the protein. Digestion of phosphorylase with pepsin in 5% formic acid and incubation of the digest with DL[^{14}C]-3-cystine or diethanol disulfide at pH 8.0 and 37°C promoted disulfide interchange and stabilized the thiol groups. The digest was fractionated on Sephadex G-25 with 0.05M acetic acid and each of the five gross fractions was submitted to the diagonal electrophoretic procedure. The results indicated that there is a minimum of 8 and a maximum of 9 unique half-cystine sequences in phosphorylase, which provides additional evidence that the subunits are identical or alternatively are identical subunits composed of non-identical polypeptide chains of molecular weight of less than 100,000. However, there is no reliable evidence for such a possibility.

Key words: Phosphorylase α and β; Allosteric transitions; Molecular symmetry; Disulfide interchange; Sulfhydryl groups; Diagonal electrophoresis.

Abbreviations:

AMP, Adenosine5′-monophosphate; DANS amino acids-, 1-dimethylaminonaphthalene-5-sulphonyl amino acids; EDTA, Ethylenediaminetetra-acetate; TPCK, 1-tosylamino-2 phenylethyl chloromethyl ketone; Cya, Cysteic acid; Glucose-1-P, α-D-glucose-1-phosphate; Diethanol disulfide (2-hydroxyethyl disulfide; or 2,2′dithiodiethanol).

Enzymes

Rabbit sketetal muscle glycogen phosphorylase α or b (α-1, 4-glucan: ortho-phosphate glycosyl- transferase, EC 2.4.1.1.); trypsin (EC 3.4.21.4); α-chymotrypsin (EC 2.4.21.1); α-lytic protease (EC 3.4.4.1).

Introduction

Glycogen phosphorylase is involved in the regulatory mechanism of glycogen metabolism in muscle, and has been used extensively as a model system of studying allosteric transitions[1,2] and the structure- function relationship of muscle enzymes[3,4]. Phosphorylase a and b have previously been shown to consist of four and two subunits respectively, each having a molecular weight of approximately 100,000[5-10]. In a previous communication[11] it was shown that there is a minimum of eight and a maximum of nine unique half-cystine sequences in each of the apparently identical subunits of phosphorylase. The sulphydryl groups have frequently been considered *inter alia* as constributing appreciably to the maintenance of protein structure. Accordingly, previous work from this laboratory has presented evidence indicating that two of these thiols was very reactive and can be alkylated without loss of activity. Another two thiol groups contribute to the stability and maintenance of the quanternary structure as well as to the enzymic activity of phosphorylase[12-16].

Additional evidence is support of the concept that the subunits of phosphorylase may be chemically identical has come from several lines of investigation including the elucidation of the structure of a unique octatriacontapeptide sequence involved in the binding of puridoxal-5 phosphate[17] and originating from the COOH-terminal polypeptide (mol. wt. 70,000) chain[9], and a unique [^{32}P]-labelled phosphoserine tetradecapeptide[18] that was derived from the NH_2- terminal (mol. wt. 30,000) end[9] of the enzyme. The isolation of a unique peptide involved in the binding of AMP has also been reported[19].

The present paper extends the work reported in an earlier communication[11] and describes the methodology developed for the isolation and characterization of all thiol sequences from peptic digests of phosphorylase in order to establish the total number of unique thiol groups. It also documents the complete sequence of these unique half-cystine peptides. The rationale was that, in the event that a total of 9 unique half-cystine sequences were found, it would indicate that the molecule consists of identical subunits of molecular weight of 97,412. In the event that the subunits are nonidentical, the value would be expected to exceed 9. Thus, the number actually found should provide an experimetal chemical basis for preferring one of these two hypotheses.

Materials and Methods

Materials

Crystalline phosphorylase b was prepared as described previously[13] except that prior to use, the crystals were collected by centrifugation (20,000Xg.), dissolved in 20mM sodium β-glycerophosphate, 1.5mM 2-mercaptoethanol and 1.5mM EDTA buffer pH 6.8 at 38°C. The protein was then passed through a Sephadex G-25 column (40 × 2.5 cm.) previously equilibrated with 1.5mM 2-mercaptoethanol and 1.5mM EDTA, pH 6.8. Protein concentration was deter-

mined from the absorbance at 280 nm with a value for $E^{1\%}_{1\,cm}$ of 13.2[20]. Enzyme activity was determined as previously described[13].

Rabbit liver glycogen, purchased from Sigma Chemical Company, St. Louis, Mo. was routinely purified through a Dowex 1-X2 chloride column. Glucose-1-phosphate and AMP were also purchased from Sigma. DL-[^{14}C]-3-cystine (17mCi/mmole), a product of Service Molecules Marquees, Fabrique per CEA-France, L-cystine and DANS-amino acids were purchased from Calbiochem, La Jolla, California. Diethanol disulfide was obtained from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. *N*-Ethylmorpholine (practical) purchased from Eastman Organic Chemicals, Rochester, New York, was redistilled (*b.p.* 134.0°C) prior to use. Sephadex G-25, fine bead type, and Blue Destran 2000 were obtained from Pharmacia (Canada) Ltd., Dorval, Quebec. Bovine pancreatic trypsin treated with TPCK to inhibit contaminant chymotryptic activity[21], and procine pepsin were purchased from Worthington Biochemical Corporation, Freehold, New Jersey. An α-lytic protease preparation from *Myxobacter 495*[22] used in these stydies was kindly provided by Dr. D.R. Whitaker (Department of Biochemistry, University of Ottawa, Ottawa, Ontario). All other chemicals were reagent grade and were used without futher purification.

Methods

Preparation of Disulfide Derivatives:

(a) *Digestion of Protein with Pepsin.* Freshly prepared phosphorylase b (1.0 to 1.5g) dissolved in 1.5mM 2-mercaptoethanol and 1.5mM EDTA solution (pH 6.8, 20.0ml) was rapidly diluted to a concentration of 10mg/ml with sufficient water and 9% (*V/V*) formic acid and was digested with pepsin (ratio of pepsin to protein, 1:10) in 5% formic acid at 37°C for 24h. Control experiments carried out with pepsin alone gave no indication of peptides that derived from its autolysis.

(b) *Disulfide Interchange Reactions.* The protein digest in 5% (*V/V*) formic acid was then adjusted to pH 8.0 with concentrated NH_4OH, and exposed to an excess (50-fold) of either DL-[^{14}C]-3-cystine or diethanol disulfide to promote disulfide interchange. In a typical example to illustrate the method, DL-[^{14}C]-3-cystine in 0.2mCi (17mCi/mmole; IM HC1) quantities was sufficiently diluted with L-cystine to give a radioactivity of 1.6μCi/mmole (26.3 ml. 1M HC1) and then was added to 0.79g of the peptic digest (79.0ml). The solution was incubated for 17h at 30°C and as a result, each of the available sulphydryl groups was converted to a mixed disulfide. The reaction was terminated by adjusting the pH to 2.0 with 99% (*V/V*) formic acid, and the flocculant precipitate attributed mainly to denatured pepsin and excess cystine was removed by centrifugation. The peptic digest was then concentrated by lyophilization. Diethanol disulfide was used in a similar way.

Peptide Purification Procedures:

(a) *Group Separation of Peptides on Sephadex G-25.* In each case initial fractionation of the peptic digests was carried out by gel filtration on Sephadex G-25 columns (195cm × 5.0cm) equilibrated with 0.05M acetic acid, the samples being applied in 25ml (250mg) of the same solvent. The flow was maintained at 180ml/h and the effluent was collected in 15.0ml fractions. The fractionation was monitored as before[12] by absorbancy measurements of the effluent fractions at 280 nm and 260 nm and by radioactivity counting. The effluent fractions were pooled as shown in Fig. 1-2 and concentrated by lyophilization.

(b) *The Diagonal Purification Procedure.* Each of the five pooled fractions so obtained was then submitted to the diagonal electrophoretic procedure of Brown and Hartley[23]. Peptide samples in 5% formic acid were applied as a band (2mg/cm, 0.03 μmole/cm) on Whatman No. 3MM paper and submitted to electrophoresis at pH 6.5 (pyridine-acetic acid-water, 25:1:224 by vol.). Voltages of 80 V/cm were maintained for 40-50 min. After electrophoresis, peptides were localized on guide strips by dipping in cadmium-ninhydrin reagent[24]. A 3 cm band from the preparative sheet containing the separated components was cut parallel to the direction of electrophoresis at pH 6.5, and exposed to performic acid vapours (19.0 ml of 98% (w/w) formic acid and 1.0 ml 30% hydrogen peroxide) in a dessicator for 2 h. When dry, the oxidized strip was stitched on a sheet of Whatman No. 3MM paper and resubmitted to electrophoresis (pH 6.5) at right angles to the original direction. After electrophoresis, the position of each of the cysteic acid peptides was revealed by staining with cadmium-ninhydrin reagent[24] and by radioactivity counting on a Nuclear-Chicago Actigraph III strip scanner with 4Π geometry. Tryptophan-containing peptides were located either with Erlich's reagent[25] on separate guide strips before oxidation or by their strong fluorescence on paper following oxidation. On such a diagonal peptide «map», each half-cystine peptide was found off the diagonal as a cysteic peptide. In the case where DL-[^{14}C]-3-cystine was used in the original disulfide interchange reaction, each cysteic acid peptide was associated with a radioactive cysteic acid vertically in line with it.

(c) *Isolation of Cysteic Acid Peptides.* After location of the cysteic acid peptides on such a diagonal peptide «map» the corresponding bands from the original electrophoregram were cut and oxidized and the strip stitched to a new sheet of paper for electrophoresis at pH 6.5. Further purification of the bands of cysteic acid peptides was frequently necessary and this was accomplished by electrophoresis at pH 1.8, 3.5 and 6.5. The peptides were normally eluted from the paper with water.

Further Proteolytic Digestion of Peptides:

Some of the peptic peptides were submitted to further digestion with TPCK-treated trypsin or α-lytic protease. Usually, 0.20 to 0.50 μmol/ml of peptide dissolved in 0.05M N-ethylmorpholine-acetic acid buffer, pH 8.0, was incubated with the proteolytic enzyme (ratio of trypsin or α-lytic protease 1:50

or 1:100) at 37°C for 5 h. The degree of digestion was judged by separating the products by paper electrophoresis at pH 6.5 or by amino acid analysis of the isolated peptide fragments.

Determination of Amides of Aspartic and Glutamic Acids:

In most cases, the assignment of amides was based on behaviour of the parent peptides of paper electrophoresis at pH 6.5[26]. However, if more than one possible amide occurred in a given peptide, the mobilities relative to aspartic acid of the peptides obtained before and after successive removal of residues by Edman degradation were compared[26].

Amino Acid Analysis:

Peptide samples were hydrolyzed under vacuum in constant boiling HCl at 110° for 22 h in sealed evacuated tubes (10 × 75 mm) with the usual precautions described by Moore and Stein[28]. Amino acid analyses were done on a Beckman Spinco Model 120C or 120B amino acid analyzer.

N-terminal and Sequence Analyses of Peptides:

N-terminal and sequence analyses were performed by methods previously described[29-30]. DANS amino acids were identified by electrophoresis at pH 4.38 combined with the thin-layer chromatography or polyamide layers according to Woods and Wang[31]. For the confirmation of some sequences, the subtractive Edman degradations were also carried out under conditions similar to those employed by Dopheide et al.[27].

Peptide Nomenclature:

The sequence of purification steps for the isolation of half-cystine peptic peptides was the following.

(a) Gel-filtration was carried out on Sephadex G-25; five fractions were designated I to V.

(b) Each fraction was then submitted to the diagonal procedure[23] and was assigned a capital letter from A to J in alphabetical order to indicate the relative mobility of the peptide on first dimension electrophoresis at pH 6.5. The most basic peptides of the digests had designations I-A and I-B, those from band I-C were neutral, and the acidic peptides were designated I-D to I-J.

(c) Finally electrophoresis was carried out at pH 6.5 or 1.8 after performic acid oxidation. Each cysteic acid peptide was assigned an Arabic number in consecutive order, the most basic peptide of the digest having the lowest number. The products of peptides subsequently digested with trypsin were designated by the letter T, or those degraded by α-lytic protease [22] were designated by the letters α-LP. An Arabic number was assigned to each of the products to indicate the relative mobility of the peptide on electrophoresis at pH 6.5, the most basic peptide of the digest having the lowest number.

Results

Isolation of the Unique Cysteic Acid Peptides

To stabilize the thiol groups during isolation, the digest was exposed to an

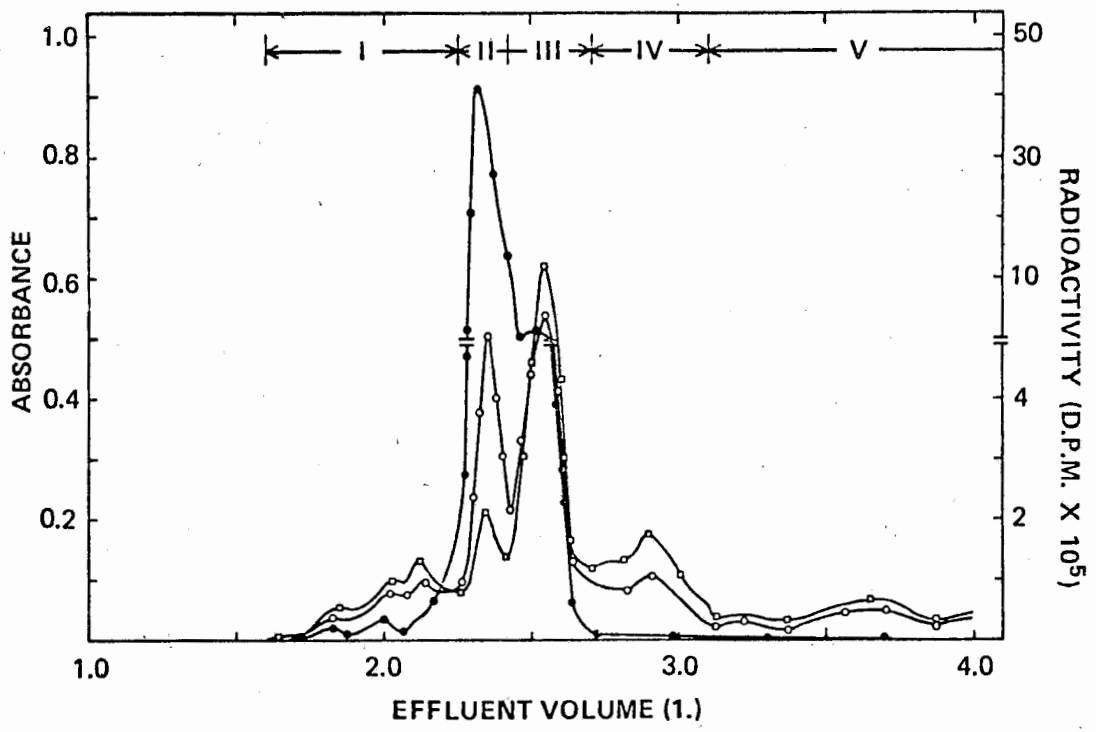

FIG. 1. *Preparative fractionation of DL-[^{14}C]-3-cystine interchanged peptic digest (0.25 mg) of phosphorylase b on Sephadex G-25.* The column (195×0.5 cm) was equilibrated with 0.05M acetic acid at 180 ml/h. The effluent was monitored for radio-activity (●——●) and absorbancy measurements at 280 nm (○——○) and at 260 nm (□——□). Fractions I to V were pooled as indicated by the vertical bars and arrows.

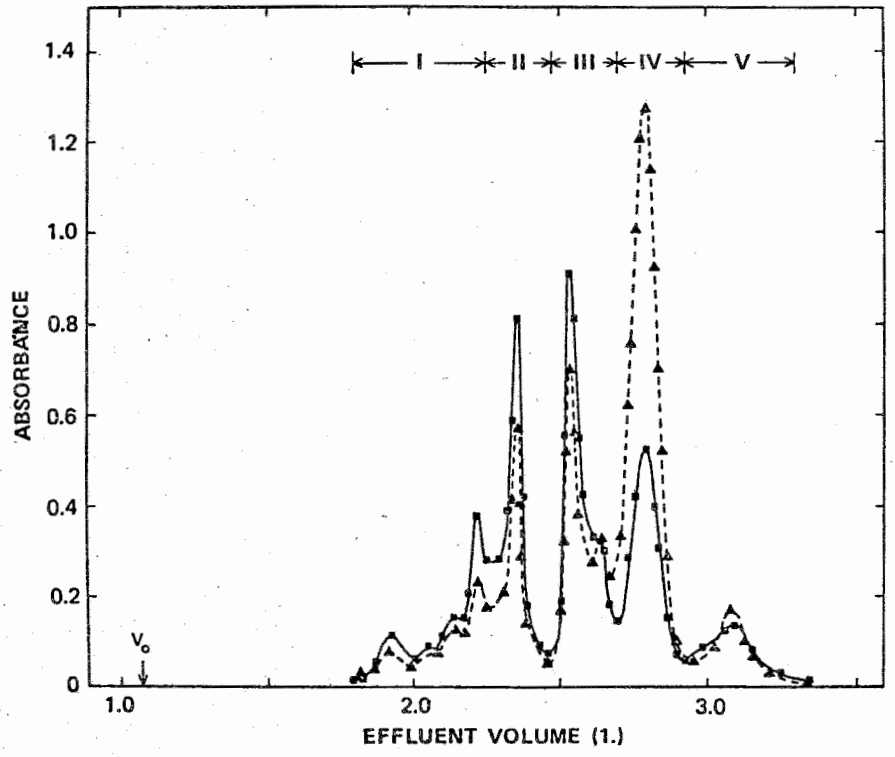

FIG. 2. *Preparative fractionation of diethanol disulfide interchanged peptic digest (0.25 mg) of phosphorylase b by get filtration on Sephadex G-25.* The column (195×5cm) was operated at 180 ml/h with 0.05M acetic acid. The effluent was monitored for absorbancy at 280 nm (■——■) and at 260 nm (▲——▲). Fractions I to V were taken as indicated by the arrows.

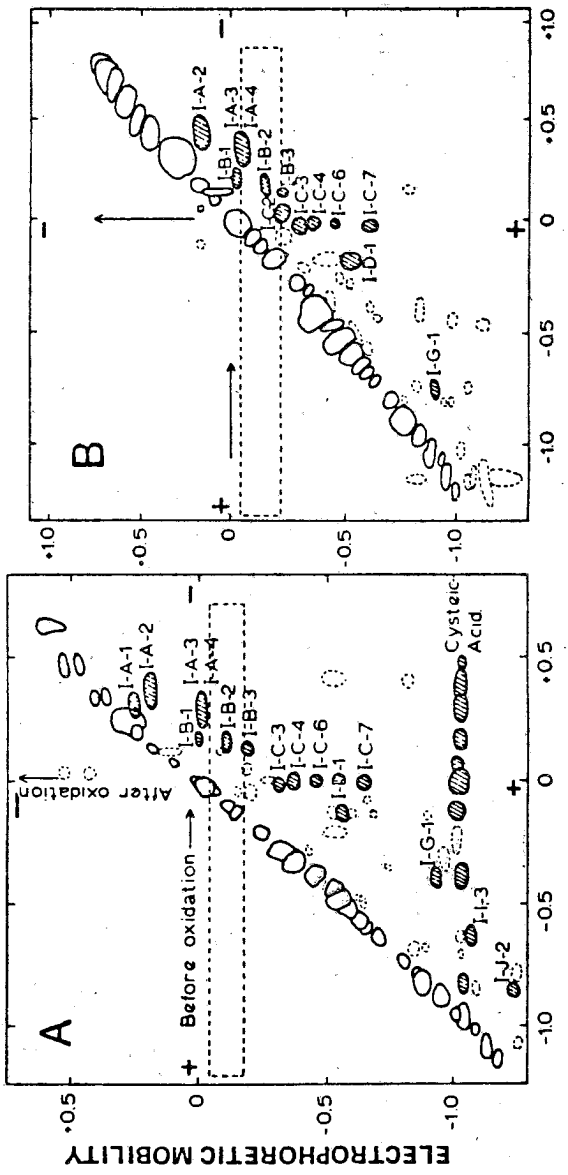

FIG. 3. *Diagonal peptide 'maps» of DL-[^{14}C]-3-cystine (A) and diethanol disulfide (B) interchanged peptic digest of phosphorylase b of fraction I.* Electrophoresis was at pH 6.5 in both dimensions. The major cysteic acid peptides are hatched. The conditions and system of nomenclature of the peptides is described in the text.

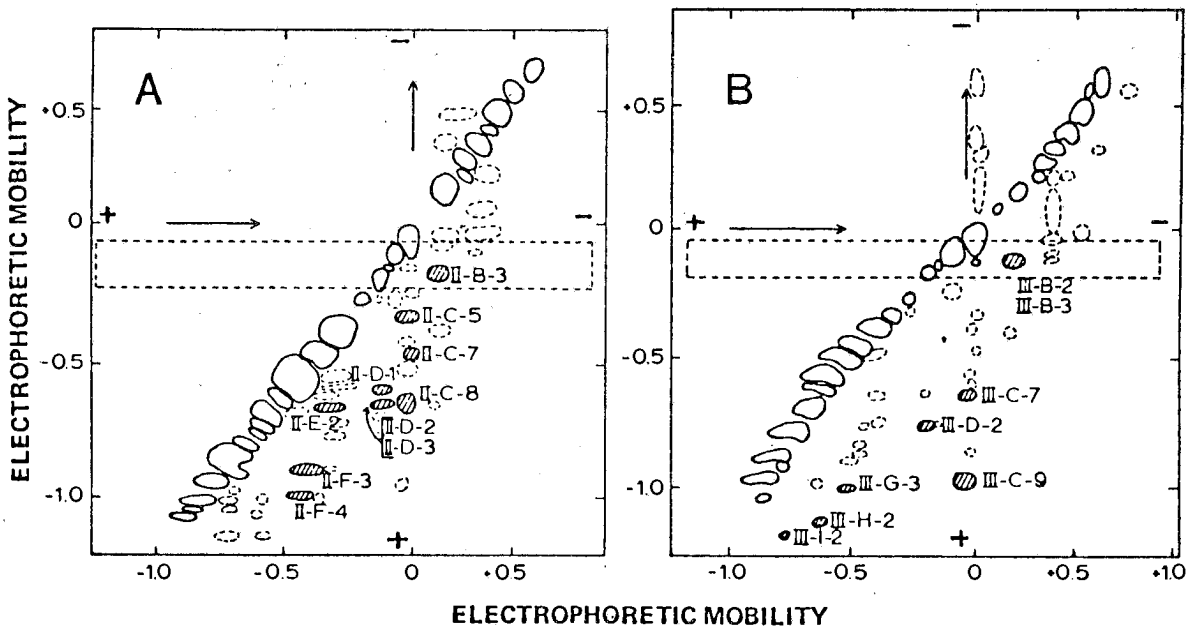

FIG. 4. *Diagonal peptide «maps» of diethanol disulfide peptic digest of phosphorylase b of fractions II (A) and III (B).* Electrophoresis was at pH 6.5 in both dimensions. The cysteic acid peptides are hatched. The conditions and system of nomeclature of the peptides is described in the text.

TABLE I. *'Amino acid composition of half-cystine peptic peptides isolated from fraction I (The values are expressed as Mole ratios)*

Amino Acid	Constituent Amino Acid Residues Per Molecule of Peptide													
	I-A-1	I-A-2	I-A-3	I-A-4	I-B-2	I-B-3	I-C-2	I-C-3	I-C-4	I-C-6	I-C-7	I-D-I	I-G-I	I-I-3
Lysine	1.05	0.92	0.97	0.85	0.92		0.79	1.00	1.00					
Histidine						0.97	1.05							
Arginine	1.05	0.93					0.66			0.98				
Cysteic Acid	0.90	0.93	0.99	1.02	1.00	0.97	1.00	0.95	0.99	1.02	0.98	0.95	1.05	0.87
Aspartic Acid	0.90	1.19			0.97	0.97	3.11			1.19		1.07	1.98	1.90
Threonine	1.21		0.89	1.71			0.74							
Serine	0.90					1.06	1.69							
Glutamic Acid		1.19			1.00		0.79	2.20	1.98					1.91
Proline	0.75						1.85					0.93		
Glycine	1.96				2.00	2.11	3.59			1.02		1.05		
Alanine	1.21		1.07	1.53		2.03	3.17				2.02		0.97	1.03
Valine	1.81		0.96	1.88		0.89	2.27	1.20	1.03					
Methionine Sulfone	1.21													
Isoleucine					0.95	1.06	1.27							
Leucine		1.84										1.00		
Tyrosine		0.79						0.75						
Phenylanine	0.90													
No. of Residues	13	8	5	7	7	10	22	6	5	4	3	5	4	6
Mobility at pH 6.5 (m)	+0.30	+0.25	0.00	0.00	−0.14	−0.25	−0.25	−0.35	−0.40	−0.52	−0.69	−0.63	−0.91	−1.05
Mobility at pH 1.8 (m')	0.95	0.81	0.63	0.54	0.83	0.67								
N-terminal		Tyr	Val	Val	Asn	Cya		Tyr	Val	Gly	Ala	Cya	Asn	Asn
Percentage Yield	0.5	6.3	24.4	3.2	14.0	9.0	3.3	14.0	18.9	18.9	16.8	36.4	8.1	1.4

TABLE II. *Amino acid composition of half-cystine peptic peptides isolated from fractions II to V (The values are expressed as Mole ratios)*

Amino Acid	Constituent Amino Acid Residues Per Molecule of Peptide																			
	II-B-3	II-C-5	II-C-7	II-C-8	II-D-1	II-D-2	II-D-3	II-E-2	II-F-3	II-F-4	III B-2	III-B-3	III-C-7	III-C-9	III-D-2	III-G-3	III-H-2	III-I-2	IV-V-2	V-C-1
Lysine	0.96	1.19									1.15									
Arginine			0.86									1.35								
Cysteic Acid	0.88	0.82	0.94	1.06	0.61	1.06	0.89	1.03	0.95	0.81	0.88	1.20	1.05	1.00	1.03	0.82	1.02	1.14	0.97	1.40
Aspartic Acid	1.05	1.19	1.15		1.19	1.13	0.91		2.02	1.37	1.10	1.12			0.96	2.35	2.03	1.96		
Serine							0.91			1.00		0.98					0.88	0.78	0.81	0.85
Glutamic Acid	2.10	2.76									1.51	2.92				1.63	0.98	3.02	0.97	0.72
Glycine	2.01	0.82	1.05		1.19						2.41	3.52					1.07	1.10	1.12	0.98
Alanine		0.82		1.94		0.81	1.28	1.97	1.03	0.81			1.95			1.29				
Proline					1.02															
Valine		0.90																		
Phenylanine																0.91				
Isoleucine	0.99										0.95	0.90								
Leucine					0.94															
Tyrosine		0.52																		
Tryptophaan[a]	+										+									
No. of Residues	9	10	4	3	5	3	4	3	4	4	8	11	3	1	2	7	6	8	5	4
Mobility at pH 6.5	−0.18	−0.37	−0.48	−0.67	−0.63	−0.68	−0.71	−0.72	−0.94	−1.03	0.15	0.16	1.06	0.67	−0.78	−1.10	−1.26	−1.12	−0.67	−0.80
Molecular Weight	1072.1	1178.2	804.8	299.2	533.0	343.2	431.2	299.2	464.0	546.4	943.0	765.7	299.2	157.0	272.1	864.7	661.5	919.8	577.5	430.3
N-terminal	Asx	Tyr	Gly	Ala				Ala	Asx						Asx	Glx				
Percentage Yield	0.8	0.4	2.5	9.6	0.2	0.14	0.9	0.4	6.2	1.5	2.7	0.5	0.9	2.9	3.5	0.9	1.1	0.5	0.01	0.02

[a]The presence of tryptophan in a peptide was inferred by positive reaction in the Ehrlich test.

TABLE III. *Amino acid composition of tryptic and α-lytic protease peptides originating from tryptic or α-lytic protease digestion of peptic peptides of phosphorylase b*

	Amino acid composition (Values are expressed as Mole ratios)													Electrophoretic mobility at pH 6.5 (m)	N terminal	Molecular weight
	Cya[a]	Lys	His	Arg	Asp	Thr	Ser	Glu	Gly	Ala	Val	Ile	Leu	Tyr			
I A 2	0.92	0.92		0.92	1.19			1.19					1.84	0.79	+0.25	Tyr	1074.2
I A 2T1				1.00											+0.96	Arg	174.2
I A 2T2		1.00		1.00										0.59	+0.88	Tyr	467.6
I A 2T 2T4		1.00												0.50	+0.59	Tyr	302.4
I A 2T11	1.09				1.03			0.86					2.10		0.49	Gln	627.6
I A 3	0.99	0.97				0.89				1.07	0.96				0.00	Val	556.6
I A 2T1		1.05									0.95				+0.70	Val	245.3
I A 3T2	1.02					0.95				1.02					0.69	Thr	329.2
I A 4	1.02	0.85				1.72				1.53	1.88				0.00	Val	756.8
I A 4T2		1.00				0.90					2.10				+0.44	Val	545.6
I A 4T6	1.02					0.95				1.02					0.67	Thr	329.2
I B 2	1.00	0.92			0.97			1.00	2.00			0.95			0.14	Asn	754.8
I B 2T1		0.96			0.96			1.07							+0.47	Asn	388.5
I B 2T2	0.99								2.07			0.94			0.57	Ile	266.3
I B 3	0.97		0.97		0.97		1.06		2.10	2.02	0.89	1.06			0.25	Cya	954.9
I B 3αLP1			1.04				0.88			1.04	1.04				+0.20	Ser	412.5
I B 3αLP2			0.75							1.23	1.04				+0.07	His	325.4
I B 3αLP4					0.99				1.01						0.18	Asn	189.2
I B 3αLP5	1.09								1.09	1.09		0.73			0.67	Cya	398.3
I B 3αLP6	0.98								0.98	1.07		0.98			0.70	Cya	398.3
I B 3αLP7	0.73						0.41		0.76	1.54		1.28			0.74	Cya	481.4
I C 3	0.95	1.00						2.20			1.20			0.75	0.36	Tyr	804.8
I C 3T1		1.04									1.30			0.65	0.50	Tyr	442.0
I C 3T5	0.93							2.06							1.03	Cya	412.3
I C 4	0.99	1.01						1.98			1.03				0.40	Val	641.6
I C 4T1		1.00									1.00				+0.69	Val	245.3
I C 4T5	0.96							2.04							1.03	Cya	412.3

[a]Cya. represents cysteic acid.

TABLE IV. *Summary of the number and amino acid sequence of half-cystine peptides from rabbit muscle phosphorylase* b.

No. of Unique Sequence	Sequence	Yield
1 I-A-3 I-A-4	Val-Thr-Val-Lys-Thr-Cya-Ala (T)	27.6
2 I-B-2, II-B-3 III-B-2	Asn-Gln-Lys-Ile-Cya-Gly-Gly(Trp, Gln) (T)	14.0
3 I-C-3, I-C-4 II, C-5	Tyr-Val-Lys-Cya-Gln-Glu (T)	32.9
4 I-C-7, II-E-2 III-C-7	Ala-Cya-Ala	36.4
5 I-C-6, II-C-7 II-C-8	Gly-Cya-Arg-Asp	16.8
6 I-G-1, I-I-3 II-F-3	Asn-Ala-Cya-Asp-Glu	9.5
7 I-A-2	Tyr-Lys-Arg-Gln-Leu-Asn-Cya (T) (T)	6.3
8 I-B-3, I-C-2 8 I-B-3-, I-C-2	α-LP α-LP α-LP Cya-Ile-Ala-Gly-(Ser-His-Ala-Val)-(Asn-Gly) (α-LP) (α-LP)	12.3
9 I-D-1, II-D-1	Cya-Asp-Pro-Gly-Leu	24.7

Definition of symbols:

Cya, represents cysteic acid.

—→ indicates quantitative amino acid analysis.

—→ represents N-terminal analyses by the dansyl-Edman method.

—→ residues marked with an arrow were determined by the substractive Edman method.

T and α-LPO represent points for enzymic cleavage of the cysteic acid peptides by trypsin and α-lytic protease respectively.

excess of either DL-[^{14}C]-3-cystine or diethanol disulfide for 17h. As a result, each of the available sulphydryl residues was converted to a mixed disulfide. After adjustment to pH 2.0, the digest from the DL-[^{14}C]-3-cystine disulfide interchange experiment was fractionated on a column of Sephadex G-25. The elution profile obtained and the five pooled fractions, labelled I to V, are shown in Fig. 1. The recoveries from the gel-filtration column of the radioactive peptide were quantitative as judged by radioactive measurements. Although most of the sequence work reported in this paper was done from cysteic acid peptides isolated from fraction I, it was found that fractions II and III were contaminated with excess labelled cystine (Fig. 1) which interfered with subsequent purification attempts by paper electrophoresis. For this reason, additional peptic digests of phosphorylase b were disulfide interchanged with diethanol disulfide and fractionated on Sephadex G-25 shown in Fig. 2. In this case Fraction I yielded many of the same cysteic acid peptides which were found in similar positions in both diagonals (Fig. 3), and the results which are presented in Tables I to III, have been combined for simplicity of presentation. A disadvantage in the unlabelled diethanol disulfide reaction was that the final yields of purified peptides were low.

The pH 6.5/6.5 diagonal peptide «map» of Fraction I from the DL-[^{14}C]-3-cystine experiment is shown in Fig. 3A, and it was used as a guide for the subsequent selective purification of cysteic acid peptides by high voltage electrophoresis at pH 6.5, 1.8 and 3.5 (see Methods). (see Methods). As may be seen in Fig. 3A, each of the half-cystine peptides (hatched) was found off the diagonal and was associated with a radioactive cysteic acid residue vertically in line with it. The diagonal peptide «map» of fraction I that originated from the diethanol disulfide interchanged peptic digest of phosphorylase b is shown in Fig. 3B. Although some differences were noted, the cysteic acid peptides were found in similar positions in both diagonals (Fig. 3). The pH 6.5/6.5 diagonals of fractions II and III originating from the diethanol disulfide interchange experiments are shown in Fig. 4. Each cysteic acid peptide thus located was isolated from the preparative sheets after performic acid oxidation followed by paper electrophoresis at pH 6.5, 1.8 and 3.5, in which case very pure peptides were obtained.

Amino Acid Sequence of Half-cystine Peptides

The amino acid composition, N-terminal determination, mobilities at pH 6.5 and percentage yields of the half-cystine peptic peptides of phosphorylase b so isolated from fractions I to V are summarized in Tables I and II. From their amino acid composition and N-terminal determinations (Tables 1 and 2) it became apparent that several peptides were larger varieties of the same sequence. Those peptides isolated in sufficient yields were grouped and labelled from 1 to 9 unique sequences[11] and they were then sequenced in parallel by the dansyl-Edman method[29] as follows:

Sequence No 1. As may be seen in Fig. 3A, the peptides designated 1-A-3 and 1-A-4 were cross contaminated, and upon further purification by electrophoresis at pH 1.8 and 3.5 had the composition shown in Table I. Both peptides had N-terminal valine. This information, and the amino acid analysis data suggested that peptide A-1-4 was a larger variety of A-I-3 in that it contained one additional residue of valine and threonine. These peptides were digested with trypsin (ratio of trypsin to peptide, 1:50) and the reaction products were separated by electrophoresis at pH 6.5 as two major bands. The amino acid composition of the tryptic peptides are shown in Table III and the amino acid sequences, determined by the dansyl-Edman method[29], are shown in Table IV. These results clearly establish that peptide I-A-4 is the same as A-I-3, except that it is a larger variety of number 1 unique half-cystine sequence.

Sequence No. 2. Similarly peptides II-B-3 and III-B-2 (Fig. 4) were found to be larger varieties of peptide I-B-2 (Fig. 3) arising from multiple pepsin cleavages of the same half-cystine sequence. After purification by electrophoresis at pH 1.8, the resulting recoveries, amino acid composition, mobilities and N-terminus of these peptides are shown in Tables I and II, indicating that asparagine and not aspartic acid must be the N-terminal residue and the additional glutamic acid must also be amidated. Peptide I-B-2 was digested with trypsin and the amino acid composistion and sequence of the two tryptic peptides (I-B-2TI and I-B-2T2) so obtained are given in Tables III and IV respectively. These results are consistent with the No. 2 unique half-cystine sequence previously reported[11], except that the presence of isoleucine which was found to be in the sequence of peptide I-B-2T2, was unintentionally omitted from the previous communication[11] due to a typographical error.

The amino acid composition of peptides II-B-3 and III-B-2 (Table 2) was consistent with previous studies[12] showing an additional glutamine and tryptophan located at the C-terminus end, although this could not be considered conclusive, since the presence of tryptophan here was inferred by its strong fluroescence on paper after performic acid oxidation. This sequence has now been extended[12] and confirmed by others[15].

Sequence No. 3. The neutral peptides I-C-3 and I-C-4 were purified by electrophoresis at pH 6.5 after oxidation and were found to have similar amino acid compositions (Table I) except that peptide I-C-3 had an additional tyrosine as its N-terminal residue. Their low mobility suggested that one of the two glutamic acid residues must be amidated. Tryptic digestion of these two peptides provided additional evidence for their amino acid sequences shown in Tables III and IV. The assignment of glutamine and glutamic acid was based on the mobilities of the peptides I-C-3T5 and I-C-4T5 (Table III) relative to glutamic acid after subtractive removal of residues by the dansyl-Edman method[29]. In summary peptides I-C-3 and II-C-5 are larger varieties of peptic peptide I-C-4 whose sequence is shown in Table IV.

Sequence No. 4. Peptide I-C-7 was isolated in high purity and good yield because of its size and position in the diagonal (Fig. 3) and gave the results

shown in Table I. The sequence of this tripeptide is shown in Table IV. Although peptides II-E-2 and III-C-7 also shown in Fig. 4 are clearly identical by all criteria, their separation in three distinct peaks (Fig. 1 and 2) may be due to absorption or other unexplained effects.

Sequence No. 5. Peptide I-C-6 purified by electrophoresis at pH 6.5 (Fig. 3) had the composition, electrophoretic mobility and N-terminal summarized in Table I. This peptide was not attacked by trypsin and its sequence was determined by the dansyl-Edman procedure and confirmed by the substractive Edman method[27]. As shown in Table IV, peptides I-C-6, II-C-7 and II-C-8 are identical in sequence by all criteria (Tables I and II).

Sequence No. 6. The peptides I-G-1, I-1-3 and II-F-3 shown in Fig. 3 and 4 were oxidized and finally purified by paper electrophoresis at pH 6.5. Peptide I-G-1 had N-terminal asparagine as determined by the electrophoretic mobility of the peptide after one Edman degradation cycle. This information, and the amino acid composition suggested that all three of these peptides were identical except that peptide I-1-3 was a larger variety of I-G-1 and was shown to contain an additional glutamic acid (Tables I and II). Their amino acid sequence was determined by the substractive dansyl-Edman procedure[27] and the results are presented in Table IV.

Sequence No. 7. The peptide I-A-2 was isolated by electrophoresis at pH 1.8 and was pure as judged by its amino acid analysis data shown in Table I. Its electrophoretic mobility at pH 6.5 indicated that both acidic amino acids must be amidated, and it had an N-terminal tyrosine. Additional evidence for the N-terminus tyrosine and for the sequence of this peptide was obtained from tryptic digestion. The four product peptides (I-A-2T1, I-A-2T2, I-A-2T4 and I-A-2T11) were separated by paper electrophoresis at pH 6.5 and the amino acid composition, N-terminal analysis and mobilities of these tryptic peptides are shown in Table III. Their sequence was determined by the dansyl-Edman method, whown in Table IV, and from the electrophoretic mobilities of peptides I-A-2 and I-A-2T11 it was concluded that both acidic amino acids were amitated.

Sequence No. 8. The decapeptide I-B-3, isolated from band I-B by electrophoresis at pH 6.5 and 1.8, gave a distinct yellow to orange colour with cadmium-ninhydrin stain, which is consistent with cysteic acid as the N-terminal residue, also found by the dansyl-Edman method. From its low mobility and amino acid composition it was concluded that asparagine rather than aspartic acid was present. Since only four steps of the dansyl-Edman procedure were possible, its sequence was also studied by digestion with α-lytic protease (ratio of enzyme to I-B-3, 1:50). The peptides produced, shown in Table III, were purified by electrophoresis at pH 6.5 and their sequences determined. The low positive mobility of I-B-3αLP2 compared to I-B-3αLP1 is attributable to a lower pK for imidazole when histidine is in the N-terminal position of a peptide, and the low positive mobility of I-B-3αLP4 can be attributed

to an abnormally low pK for the α-amino group of this peptide, an observation which is common with peptides containing N-terminal asparagine. Since it was not possible to obtain the overlapping peptide I-B-3αLP7 in sufficient quantity for sequence determination and because its amino acid composition was equivocal, it was difficult to determine the C-terminus peptide and to align I-B-3αLP1 and I-B-3αLP4 in order. The partial sequence of I-B-3 is therefore shown in Table IV.

Sequence No 9. The peptide I-D-1 purified by electrophoresis at pH 6.5 had N-terminal cysteic acid. Its position in the first dimension electrophoresis at pH 6.5, and mobility after oxidation (Fig. 3) together with the amino acid composition indicated that there were two negative charges, consistent with one aspartic acid and one cysteic acid described in Table I. Its sequence determined by the dansyl-Edman method showed that this pentapeptide had the structure shown in Table 4. Peptide II-D-1 (Table II) is clearly identical to I-D-1 of fraction 1.

The remaining minor peptides summarized in Table II were found to correspond to varieties of the sequences reported in Table IV. The only minor peptides isolated in any digest whose composition was incompatible with the known sequences were III-H-2, III-1-2, IV-C-2 and V-C-1. These may be varieties of either I-B-3 or I-D-1 or they may arise from peptic clevages of protein contaminating certain phosphorylase preparations.

Discussion

The experiments described in this paper for the isolation and determination of the numbers and sequences of the unique half-cystine peptides in phosphorylase were designed to derive information on the basic structural subunit of the enzyme. For the isolation of these peptides the diagonal electrophoretic procedure was originally employed. However, this method was not directly applicable to the elucidation of sufhydryl sequences of phosphorylase because of the high molecular weight of the enzyme and the instability of its thiol groups during the electrophoretic separation procedure. It was therefore decided to disulfide interchange the peptic digest of phosphorylase b with DL-[^{14}C]-3-cystine. This method had the advantages that the thiol groups were protected from oxidation during the isolation procedures and that these diagonal techniques were greatly simplified. The disulfide diethanol reagent had the advantage over DL-[^{14}C]-3-cystine in that it was completely solube and did not interfere with subsequent purification procedures.

Peptic digestions of phosphorylase performed in this work were found to produce families of peptides arising from the same sequence. This complicated somewhat the diagonal procedure for the isolation of all cysteic acid peptides in good yields (Tables I and II). For example, the low recoveries of the unique half-cystine sequences, ranging from 6.0 to 36.0 per cent of the theoretical are accounted for by the low specificity of peptic cleavage, the large number of

purification steps by paper electrophoresis and the adsorption to and elution from paper. It may also be noted that in the unique half-cystine sequences, numbers 7 to 9, cysteic acid is situated either in the N-terminal of C-terminal position. These results are consistent with the broad specificity of pepsin and the susceptibility of peptide bonds formed by the amino or carboxyl groups of cysteine. Further complications were encountered in situations where pepsin cleaved the susceptible peptide bond formed by trytophan at the α-amino group. Such peptides upon oxidation acquired one or two negative charges. These fluorescent tryptophan peptides found off the pH 6.5/6.5 diagonals (see Fig. 3 and 4) tended to contaminate the cysteic acid peptides to a considerable extent. These complications did not hinder the purification procedures, but because of these difficulties approximately 140 minor peptides were isolated and analyzed so that no cysteic acid containing peptide would be overlooked.

From the experimental evidence described and the results presented in Table IV, it can be concluded that there is a minimum of 8 and a maximum of 9 unique half-cystine sequences per monomer of phosphorylase b, and these may be written as:

No. 1 Val-Thr-Val-Lys-Thr-Cya-Ala;
No. 2 Asn-Gln-Lys-Ile-Cya-Gly-Gly, (Trp, Gln);
No. 3 Tyr-Val-Lys-Cya-Gln-Glu;
No. 4 Ala-Cya-Ala;
No 5. Gly-Cya-Arg-Asp;
No. 6 Asn-Ala-Cya-Asp-Glu;
No. 7 Tyr-Lys-Arg-Gln-Leu-Leu-Asn-Cya;
No. 8 Cya-Ile-Ala-Gly, (Ser-His-Ala-Val), (Asn-Gly);
and No. 9 Cya-Asp-Pro-Gly-Leu.

These results provide additional evidence that the subunits of phosphorylase are identical. The possibility remains that minor differences may exist in the subunits which do not involve residues in the immediate vicinity of the half-cystines, . the ε-N-pyridoxylphosphatelysine moiety or the phosphorylated serine and AMP binding site. Nor to the present results exclude the existence of identical subunits composed of non-identical polypeptide chains of molecular weight of less than 97,412. However, there is no reliable evidence for such a possibility.

In addition to giving information concerning the subunit structure, such sequence analyses contributed significantly to the identification of those thiol residues which are reactive and those thiols associated with the maintenance of the quaternary structure and enzymic activity of phosphorylase. The evidence presented in this paper should also be valuable to the ultimate elucidation of the primary, tertinary and quaternary structure of this protein now in progress elsewhere[3,9,32-37]

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Περίληψη

Ἀλληλουχίαι θειολῶν καὶ δομὴ ὑπομονάδων τῆς φωσφορυλάσης τῶν μυῶν

Εἰς τὴν παροῦσαν ἐργασίαν περιγράφεται ἡ ἀνάπτυξις μεθόδου ὄχι μόνον διὰ τὸν διαχωρισμὸν καὶ τὸν καθαρισμὸν τῶν ὑπὸ σύνταξιν πεπτιδίων κυστεΐνης, ἀλλὰ καὶ διὰ τὴν σήμανσιν καὶ προστασίαν αὐτῶν ἀπὸ ὀξειδώσεις τόσον κατὰ τὴν ὑδρόλυσιν αὐτῶν ὅσον καὶ κατὰ τὸν διαχωρισμόν των διὰ χρωματογραφίας. Τῆς ἀναλύσεως δομῆς τῶν πεπτιδίων κυστεΐνης προηγήθησαν ὑδρολύσεις τοῦ ἐνζύμου ὑπὸ πεψίνης εἰς ἀναλογίαν 10:1 κι' εἰς διαλύματα μυρμηκικοῦ ὀξέος (5%, pH 2.0) θερμοκρασίας 35°C καὶ διαρκείας 18 ὡρῶν, ἐπώασις τοῦ πεπτικοῦ διαλύματος μετὰ ραδιενεργοῦ DL-[^{14}C]-3-κυστεΐνης, ἀλλὰ καὶ διὰ τὴν σήμανσιν καὶ προστασίαν αὐτῶν ἀπὸ ὀξειδώσεις τόσον κατὰ τὴν ὑδρόλυσιν αὐτῶν ὅσον καὶ κατὰ τὸν διαχωρισμόν των διὰ χρωματογραφίας. Τῆς ἀναλύσεως δομῆς τῶν πεπτιδίων κυστεΐνης προηγήθησαν ὑδρολύσεις τοῦ ἐνζύμου ὑπὸ πεψίνης εἰς ἀναλογίαν 10:1 κι' εἰς διαλύματα μυρμηκικοῦ ὀξέος (5%, pH 2.0) θερμοκρασίας 35°C καὶ διαρκείας 18 ὡρῶν, ἐπώασις τοῦ πεπτικοῦ διαλύματος μετὰ ραδιενεργοῦ DL-[^{14}C]-3-κυστίνης ἢ διαιθυλοδισουλφιδίου κατὰ τὴν ὁποίαν τὰ σουλφυδρυλικὰ πεπτίδια μετατρέπονται πρὸς δισουλφιρικὰ τοιαῦτα εἰς περιβάλλον pH 8,0 καὶ εἰς τοὺς 30°C διὰ χρονικὸν διάστημα 17 ὡρῶν, καὶ τέλος διαχωρισμὸς καὶ καθαρισμὸς αὐτῶν διὰ συνδυασμοῦ χρωματογραφίας διὰ στήλης (200×5 cm) Sephadex G-25 [Σχήματα 1 καὶ 2] καὶ δι' ἐπακολουθούσης διδιαστάτου διαγωνίου ἠλεκτροφορτήσεως ὑψηλῆς τάσεως εἰς pH 6,5/6,5 ἢ 1,8 καὶ 3,5 μετ' ἐνδιαμέσου ὀξειδώσεως τῶν πεπτιδίων κυστεΐνης δι' ἀτμῶν ὑπερμυρμηκικοῦ ὀξέως κατὰ Brown καὶ Hartley 23. Οὕτω ἐπῆλθε τέλειος διαχωρισμὸς τῶν ραδιενεργῶν πεπτιδίων, τῆς τάξεως τοῦ κυστεϊκοῦ ὀξέος [Σχήματα 3 καὶ 4], ἀπ' ἀλλήλων, καὶ ἐπετεύχθη ἡ ἐξακρίβωσις τῆς συστάσεως αὐτῶν [Πίνακες I καὶ II]. Τέλος δι' ἀναλύσεων τῶν τελικῶν ἀμινοξέων ὅλων τῶν ἀπομονωθέντων κυστεϊκῶν πεπτιδίων κατὰ τὴν μέθοδον Gray [29-30], γνωστὴ ὡς «Dansyl-Edman Procedure», καὶ κλιμακω-

τὴν ὑδρόλυσιν αὐτῶν ἐπετεύχθη ὁ προσδιορισμὸς τῆς σειρᾶς, καθ' ἣν τὰ ἀμινοξέα τῶν ἀπομονωθέντων πεπτιδίων εἶναι ἡνωμένα πρὸς ἄλληλα. Κατὰ τὸν ἀνωτέρω τρόπον καὶ διὰ τῆς μερικῆς χρήσεως καὶ τῆς ἐνζυμικῆς ὑδρολύσεως αὐτῶν ὑπὸ TPCK-τρυπσίνης ἢ α-λυτικῆς προτεάσης [Πίναξ III] ἐπετεύχθη ἡ βῆμα πρὸς βῆμα ἐξακρίβωσις τῆς συντάξεως ὅλων τῶν ὑπὸ μελέτην πεπτιδίων κυστεΐνης τῆς φωσφορυλάσης b [Πίναξ IV].

Ἐπὶ τῇ βάσει τῶνἀνωτέρω χημικῶν μελετῶν καὶ πειραματικῶν δεδομένων συμπεραίνεται, ὅτι ἡ φωσφορυλάση b ἐνέχει εἰς τὸ μόριον αὐτῆς κατ' ἐλάχιστον ὅριον 8 καὶ κατὰ μέγιστον 9 μοναδικὲς ἀλληλουχίες ἢ γειτονίας ἀμινοξέων ἐντοπισμένων ἑκατέρωθεν ἑκάστης ἐκ τῶν ἐννέα μοναδικῶν μορίων κυστεΐνης [Πίναξ IV], καὶ ὅτι ἑκάστη τῶν μοναδικῶν ἀλληλουχιῶν κυστεΐνης ἀφορᾶ καὶ τὰ δύο ἡμίσυ τῆς φωσφορυλάσης b. Δεδομένου περαιτέρω, ὅτι τόσον ἡ φωσφορυλάση α ὅσον καὶ ἡ b εἶναι ὀλιγομερῆ ἀλλοστερικὰ ἔνζυμα ἀποτελούμενα ἐκ τεσσάρων καὶ δύο μονομερῆ ἀντιστοίχως, τῆς τάξεως τῶν 97.412 dalton, κατέστη φανερὸν ὅτι τὰ μονομερῆ εἶναι ταυτόσημα, καὶ ὅτι συνδέονται τοιουτρόπως ὥστε νὰ κατέχουν ἰσοδυνάμους θέσεις εἰς τὴν μοριακὴν συμμετρίαν τοῦ ἐνζύμου. Γενικῶς διὰ τῆς χρησιμοποιηθείσης χημικῆς μεθόδου προσεγγίσεως τοῦ προβλήματος ἐπιβεβαιοῦται μὲ ἀκρίβειαν ἡ ἐκπεφρασμένη ταυτότης καὶ συμμετρία εἰς τὴν δομὴν τῆς φωσφορυλάσης α ἢ b κατὰ τὴν ὑπόθεσιν τῶν Jacob, Monod καὶ Changeux 4, τὰ δὲ πειραματικὰ δεδομένα ἀποκλείουν τὴν ὕπαρξιν μὴ ταυτοσήμων μονομερῶν μικροτέρου μοριακοῦ βάρους.

Ἐπὶ τῇ βάσει τῶν ἀνωτέρω πειραματικῶν δεδομένων 11 διευκρινίσθη κατὰ πρῶτον ἡ ταυτότης καὶ ἀτομικότης τῶν δραστικῶν σουλφυδρυλικῶν ὁμάδων κυστεΐνης τῆς φωσφορυλάσης b, ἤδη ὡς ὑπ' ἀριθμὸν 2 καὶ 5 ἀλληλουχίας πεπτιδίων κυστεΐνης [Πίναξ IV], καὶ αἱ ὁποῖαι εὑρισκόμεναι πλησίον ἢ εἰς τὴν ἐπιφάνειαν τοῦ ἐνζύμου ἐπιδροῦν ἐκλεκτικῶς ἐπὶ τῆς διαμορφώσεως καὶ ἀλλοστερικῶν ἰδιοκτήτων τοῦ ἐνζύμου 12-16, καὶ δεύτερον προωθήθη ἡ σύνταξις τοῦ μορίου 9, 17, 32-33, 35], καὶ ἡ δι' ἀκτίνων X ἔρευνα ἐπὶ τῆς ἐνδομοριακῆς ἀρχιτεκτονικῆς διαμορφώσεως τῶν κρυστάλλων τῆς πρωτεΐνης ὡς ἓν σύνολον 34, 36-49.

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CHANGES IN THE EXTRA PROTEIN FRACTION OF MYOFIBRILLAR PROTEINS

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Summary

Chemical and proteolytic changes occuring in the extra protein fraction of myofibrillar proteins of excised bovine skeletal muscle held 12 days at 4°C were studied. Evidence was obtained indicating that the extra protein was altered during the 12-day sampling period. Polyacrylamide-gel electrophoretograms of the 0-day extra protein samples showed one more band than those for the 12-day samples. Verification of the presence of tropomyosin and nucleoproteins in the extra protein fraction was obtained by ultraviolet absorbance data and viscometric analysis of fractions of extra protein separated by polyacrylamide-gel electrophoresis and collected by the elution convection procedure.

Key words: Extra protein fraction, elution - convection.

Introduction

Numerous studies have been concerned with the post-mortem properties and characteristics of the major muscle structural proteins (actin, myosin and tropomyosin) and with actomyosin, the rigid protein complex formed during rigor mortis. Very little data have been collected to indicate dissociation of actomyosin or the proteolytic degradation of the main structural proteins during normal aging[1]. However, results of several studies[2,3] suggest that the Z-line may disintegrate or deteriorate during aging.

To gain further knowledge about the biochemical changes occurring in post-mortem muscle, research has been conducted on other lesser known myoproteins[4,5]. The purpose of this study was to obtain information about the extra protein fraction and to investigate the possibility that it undergoes post-mortem hydrolysis.

Experimetal

Procedures of Perry and Zydowo[6] and Petropakis et. al.[7] were used to extract myofibrils from beef psoas major muscle held at 4°C for 0 and 12 days. All extraction procedures were completed at 4°C. The extracts were dialyzed against deionized water for 24 hr prior to lyophilization.

Protein Extraction

Lyophilized myofibrils were mixed with 4-5 volumes of Hasselbach-Schneider solution (0.1 M potassium phosphate buffer (pH 6.5), 0.47 M KCl, 1 mM $MgCl_2$, 0.01 M sodium pyrophosphate). The mixture was gently stirred 30 min and centrifuged at 600 X *g* for 30 min. The turbid supernatant was dialyzed against 6.7 mM potassium phosphate buffer (pH 7.2) containing 40 mM KCl (Perry and Zydowo, 1959) for 48 hr with several changes of dialysis medium. After dialysis and ultracentrifugation (40,000 × *g* for 20 min), the clear supernatant containing the extra protein was lyophilized.

Troponin was extracted from myofibrils by the procedure of Schaub and Perry 8 with subsequent suspensions being lyophilized and stored at 0°C until used. Tropomyosin B was prepared by the method of Bailey[9].

Protein Determination

Nitrogen content of the extra protein preparations was determined by the AOAC micro-Kjeldahl method and biuret procedure[10]. Ultraviolet absorption measurements at 280 and 260 nm were used to estimate protein and nucleic acid[10]. Protein content of samples used for electrophoresis was determined by the micro-Kjeldahl method.

Polyacrylamide-Gel Electrophoresis

An EC470 vertical gel electrophoresis assembly (E-C Apparatus) was used to fractionate extra protein preparations by the discontinuous technique described by Petropakis et. al.[11] except for the following modifications. Urea (5 M) and 1 mM dithiothreitol were added to all gel buffers prior to preparation of gels and to electrode buffer. Spacer buffer (62 mM Tris-48 mM HCl (pH 6.7) was used to prepare 4% spacer gels. Plug and running gels (8%) were prepared with 0.38 M Tris-48 mM HCl running buffer (pH 9.0). The electrode buffer consisted of 0.01 M Tris-39 mM glycine (pH 8.6). A «sample mixture» of 15% sucrose plus 5 M urea and a small amount of marker dye (bromophenol blue) was used to dilute samples to a protein concentration of 5 mg/ml.

Elution Convection Procedure

The elution convection technique[12,13] was employed to recover proteins separated by polyacrylamide gel electrophoresis. This procedure was performed immediately after completion of electrophoresis with an EC760 elution convection cell (E-C Apparatus) using 0.01 M Tris-39 mM glycine (pH 8.6) containing 4 M urea and 1 mM dithiothreitol as the eluting buffer. A constant potential of 20 volts (100 mA) was applied for 8-10 hr. Microcuvettes were used to measure absorbance at 280 and 260 nm of 1.5 ml fractions collected from the elution grid.

Viscosity Determinations

Viscosity measurements of a series of samples were carried out at 25°C in

0.01 M Tris buffer (pH 7.6) with Ostwald viscometers[14]. Viscosity of fractions obtained by elution convection was measured after these fractions were dialyzed against 0.01 M Tris buffer (pH 7.6) (T buffer) for 24 hr. To dialyzed fractions, denoted by their tube number, increasing quantities of troponin preparation (TN) were added to check for a possible increase in viscosity which would indicate the presence of tropomyosin in these fractions.

Dialyzed fractions were also lyophilized and the dried samples were added to tropomyosin preparations diluted to 0.025 mg protein/ml with T buffer, to check again for a possible increase of viscosity which would indicate the presence of troponin in these fractions.

The relative viscosity (n_r) of the tropomyosin samples was calculated from the formula $n_r = n/n_o = (d_o/d) \cdot (t/t_o)$ where t and t_o are the flow times of the sample and water, respectively, while d, d_o refer to densities of the sample and water. For these measurements it was assumed that $d_o/d = \delta$ remained constant. In calculating the spectific viscosity[15] of the elution convection samples ($n_{sp} = (d_o/d) \cdot (t/t_o) - 1$), it was again assumed that $d_o/d \simeq 1$ over the range of protein concentrations employed in these experiments.

Results and Discussion

Protein contents of lyophilized extra protein samples are given in Table I. The estimation of protein by the biuret method agrees relatively well with the micro-Kjeldahl results. Protein contents as estimated by ultraviolet absorption are about 40% less than those obtained with the above methods. Tropomyosin has been reported to be a component of the extra protein complex[6,16]. This protein does not contain tryptophan[17] which is the amino acid accounting for most of the ultraviolet absorption[18]. Thus, differences between the protein contents determined by the biuret or micro-Kjeldahl methods and ultraviolet absorption support the reported data about the presence of tropomyosin in the extra protein.

Salt contents of sample solutions must be reduced to an ionic strength of 0.03 - 0.05 to achieve maximum electrophoretic resolution[19]. Thus, dialysis against 6.7 mM potassium phosphate 40 mM KCl buffer (pH 7.2) proved to be a critical step in the preparation of the extra protein samples for elec-

TABLE I: *Protein contents of lyophilized extra protein samples.*

Sample	Biuret*	Micro-Kjeldahl*		UV Absorption
	(mg/g)	(%N)	(mg/g)	(mg/g)
0-day	120.0	17.82	111.4	65.1
12-day	105.9	16.80	105.0	62.0

* Means of five determinations (n=5). Differences between 0-day and 12-day samples were significant at 0.05 level of probability.

trophoresis. After determination of the protein content, lyophilized extra protein was diluted with deionized water to a protein concentration of 15 mg/ml. These solutions were dialyzed against the above buffer for 24 hr at 4°C and lyophilized once again. These samples were diluted with 3 volumes of «sample mixture» to obtain a protein concentration of 5 mg/ml which was suitable for electrophoretic analysis. Dialyses were necessary to obtain better resolution and to avoid excessive ohmic heating of gels.

In preliminary studies, electrophoretic runs of several hours duration were performed in which most of the extra protein did not penetrate the 4% spacer gel but remained at the site of sample application. This problem involving protein aggregation has been encountered in experiments involving other structural proteins by several investigators[8,20], who avoided the difficulty by adding urea to buffer systems. Dithiothreitol was used as a sulfhydryl protecting agent[8]. The inclusion of 5 M urea and 1 mM dithiothreitol and the use of electrode buffer with adequate buffering capacity greatly improved electrophoretic resolution.

Results of polyacrylamide-gel electrophoresis of the extra protein samples held 0 and 12 days are presented in Figure 1. Stained bands, corresponding to proteins having different electrophoretic properties, are designated by numbers on pattern margins. In these patterns, 9-10 different bands can be distinguished. Two faintly visible areas (labeled?) might also be interpreted as protein bands. The major difference between the 0- and 12-day samples is that band 4 is clearly visible at 0-day but not in the 12-day pattern. Also, the protein content of the extra protein preparations decreased with aging (Table I), which might be related with the disapperance of band 4 in electrophoretograms of the 12-day sample. It has been reported[4] that the amount of α-actinin from conditioned muscle which could be bound to F-actin was reduced during aging. Other studies[6] on extra protein infer that not all of the sarcoplasmic proteins were removed during washing of myofibrils prior to extraction of the extra protein. Although identical procedures were used for preparation of both 0- and 12-day samples in this study, some proteins may have remained with the 0-day myofibrils during several washings but were removed from myofibrils of the 12-day sample. It was suggested[22] that aging may result in an alteration of binding of some proteins to each other in the myofibril but no evidence of protein degradation was detected. Studies on the soluble proteins released from myofibrils indicated the presence of α-actinin and of smaller molecular weight products that could not be identified with the known myofibrillar proteins[21]. Characterization of band 4 was beyond the scope of this paper.

Fig. 2 shows the UV measurements of elution convection fractions plotted against collection tube number. Generally, peaks of elution convection patterns correspond to bands of the electrophoretograms in Figure 1. However, carryover of some proteins from one fraction to another may have occured during elution convection.

Significant amounts of tropomyosin have been reported[6] to occur in certain fractions obtained by DEAE-cellulose chromatography of extra protein. Con-

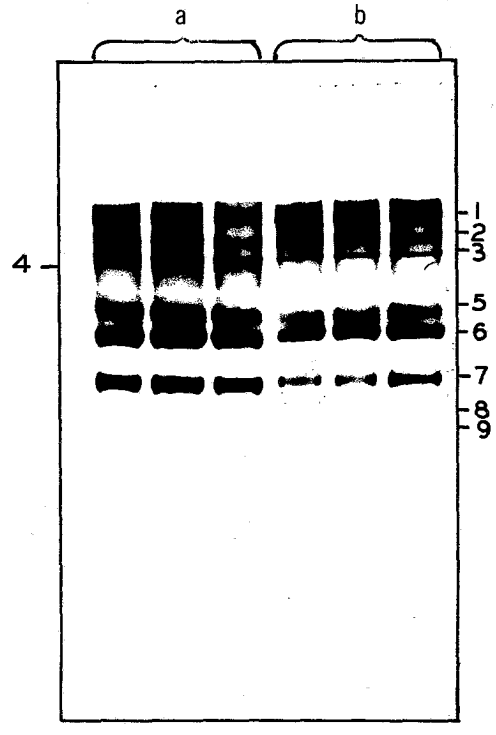

FIG. 1: *Polyacrylamide-gel electrophoretic patterns of extra protein preparations at 0- (a) and 12-days (b) post-mortem. Electrophoretic conditions: discontinuous buffer and gel systems containing 5 M urea and 1 mM dithiothreitol; 1.5 hr prerun at 200 V; and 3 hr main run at 400 V.*

current electrophoretic runs of extra protein, troponin and tropomyosin were completed to determine whether the latter two components would correspond with any of the electrophoretic bands of extra protein. These results were not conclusive although they did indicate the possible presence of tropomyosin in bands 5, 6 and 7, of the electrophoretograms in Figure 1. Presence of troponin in the extra protein electrophoretic patterns was not evident.

Further information on the above aspects was obtained by viscometric analysis of fractions separated by electrophoresis and collected by the elution convection procedure. A micromethod[14] for detecting tropomyosin utilizing the viscosity increasing action of troponin on tropomyosin was employed. An increase in viscosity was noted only in fractions of tubes 21 and 22 for both 0- and 12-day samples. This indicates the presence of tropomyosin in band 7, the fastest moving major band in Figure 1. An earlier report[16] indicated that the fastest moving main component during electrophoresis of extra protein was tropomyosin.

Using the same procedure, eluted fractions were lyophilized and added in

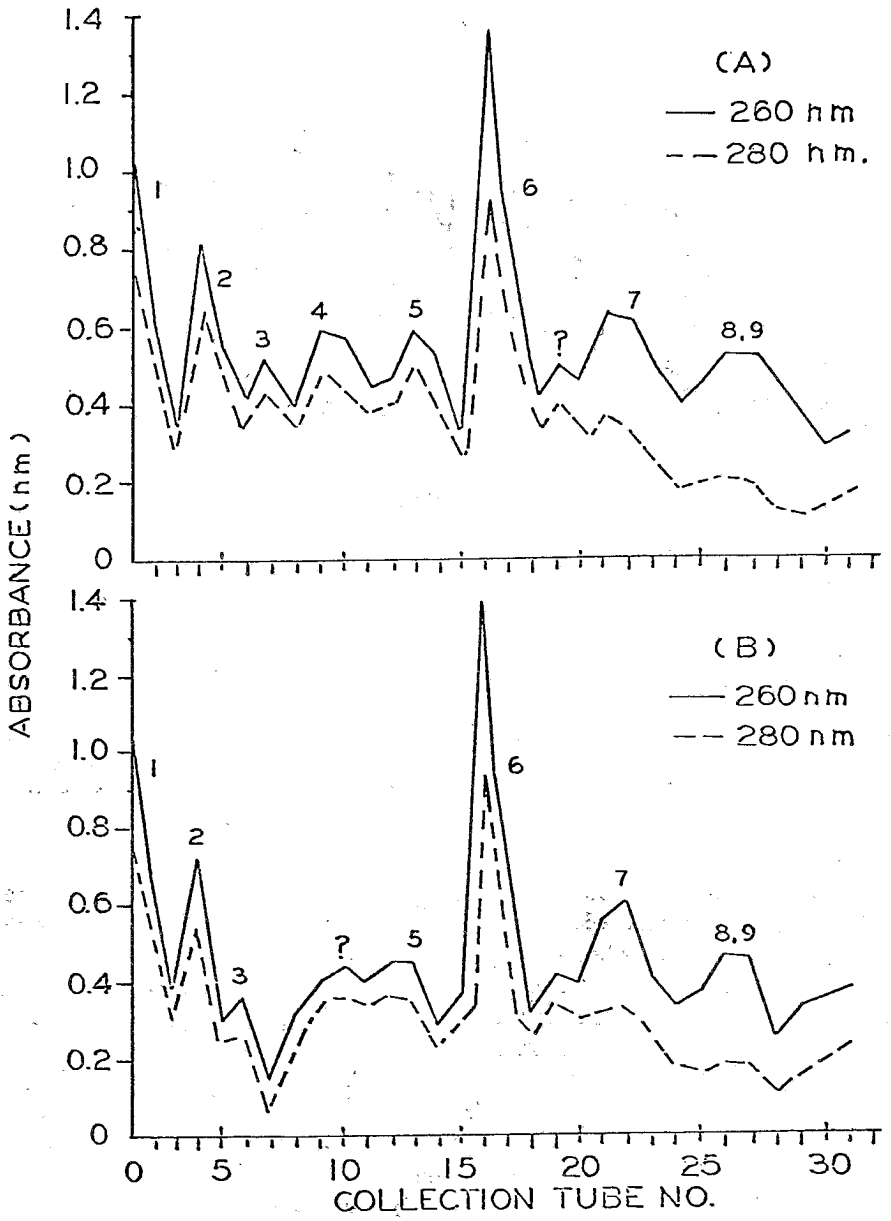

FIG. 2: *Elution convection patterns of extra protein preparations at 0- (a) and 12-days (b) post-mortem. The numbers of the peaks refer to the corresponding bands of the electrophoretic patterns of Figure 1. Ultraviolet absorbance data at 260 and 280 nm.*

increasing amounts to troponin preparations. In this case, an increase in viscosity would be indicative of troponin in the eluted fractions. No increase in viscosity was noted with any of these fractions.

The presence of nucleoproteins in extra protein was reported by Perry and Zydowo[6] in their DEAE-cellulose chromatography study of extra protein. On the basis of the ratio absorbance of 280 nm to 260 nm, elution convection patterns (Figure 2) also indicate the presence of nucleoproteins. For example, the main peaks had A_{280}/A_{260} ratios of 0.66 and 0.68 for the 0- and 12-day samples. The very low ratios of peaks 7, 8 and 9 can be attributed partly to eluted impurities which absorb more strongly at 260 nm.

Evidence obtained in this study shows that a change in electrophoric properties occurred in myofibrillar proteins with time. The appearance of band 4 in the electrophoretogram of the 0-day sample but not in the 12-day pattern can be interpreted that either this part of the extra protein was degraded or more possibly, its ability to bind to other proteins had been altered. This appears to be a significant finding when considering that changes in the binding and anchoring of myofibrillar proteins to the Z-line have been suggested[3,22] as a partial explanation of the post-mortem tenderization of muscle or meat.

Results of this study can serve as a basis for further investigations in which band 4 could be characterized with the use of SDS electrophoresis. The latter has been used with significant successs in recent studies on myoproteins. Data from the suggested studies would undoubtedly help to advance findings reported herein on certain changes occuring during the aging of meat.

Περίληψις

Μελέτη τῶν ἀλλαγῶν ὡρισμένων πρωτεϊνῶν τοῦ μυϊκοῦ συστήματος.

Εἰς τὴν ἐργασίαν αὐτὴ μελετῶνται οἱ χημικὲς καὶ πρωτεολυτικὲς ἀλλαγὲς οἱ ὁποῖες λαμβάνουν χώραν εἰς τὶς πρωτεῖνες τοῦ μυϊκοῦ συστήματος κατὰ τὴν διατήρησιν τῶν δειγμάτων εἰς τοὺς 4°C ἐπὶ 12 ἡμέρες. Ἐκ τῆς ἐρεύνης αὐτῆς προέκυψαν ἐνδείξεις ὅτι ὡρισμένες πρωτεῖνες χαρακτηριζόμενες ὡς «extra proteins» ὑφίστανται ἀλλαγὲς κατὰ τὴν δειγματοληπτικὴν περίοδον 12 ἡμερῶν. Δείγματα 0 ἡμερῶν ἐξετασθέντα μὲ κάθετον ἠλεκτροφόρησιν polyacrylamide gel ἔδειξαν τὴν ὕπαρξιν μίας ἐπὶ πλέον ἠλεκτροφορετικῆς ζώνης ἐν σχέσει μὲ τὰ δείγματα τῶν 12 ἡμερῶν. Πειραματικὰ δεδομένα ἀπὸ τὶς μετρήσεις ἀπορροφήσεως ὑπεριώδους φωτὸς καὶ ἀπὸ ἰξωδομετρικὲς ἀναλύσεις μερῶν τῶν «extra proteins» διαχωρισθέντων ἠλεκτροφορετικῶς καὶ συλλεγέντων μὲ τὴν τεχνικὴν elution - convection ἐπιβεβαιώνουν τὴν παρουσίαν τροπομυοσίνης καὶ νουκλεοπρωτεϊνῶν.

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THE POLARISABILITY AND THE NUCLEAR SCREENING CONSTANT ANISOTROPIES FOR ^{13}C AND ^{19}F IN C_6H_6, 1, 3, 5-$C_6H_3F_3$ AND C_6F_6

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Summary

Mc Weeny's SCF perturbation theory has been applied to the calculation of the polarisability and the nuclear screening constant anisotropies for ^{13}C and ^{19}F in C_6H_6, 1, 3, 5 - $C_6H_3F_3$ and C_6F_6.

The calculations were succesful in reproducing qualitatively – and in the polarisability anisotropy quantitatively – the essential features of the examined anisotropies.

Key Words: SCF perturbation theory, polarisability and nuclear screening constant.

Introduction

Mc Weeny's SCF perturbation theory[1] with its well known "economy of thinking"[2] attained by using density matrices, has been used for the calculation of the polarisability, α, and the nuclear screening constant, σ, for ^{13}C and ^{19}F in C_6H_6, 1, 3, 5-$C_6H_3F_3$ and C_6F_6.

The polarisability is a property of the molecule as a whole. All electrons contribute to it. The nuclear screening constant is mainly a property of the molecular point where the nucleus is situated, and local terms make usually predominant contributions to it. So these properties complement each other in the study of the electronic structure.[3]

The emphasis is on the anisotropies of α and σ, $\Delta\alpha$ and $\Delta\sigma$ respectively, rather than on the average values, $\langle\alpha\rangle$ and $\langle\sigma\rangle$, since the anisotropies provide a more detailed probe into the molecular structure than the average values.[4]

$\Delta\alpha$ is a property of considerable interest because of its use for the determination of molecular quadrupole moments and magnetic susceptibility anisotropies.

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In the area of ^{19}F $\Delta\sigma$ there is a great deal of confusing and conflicting data.[3] This work reexameines some of the existing data and hypotheses. In addition a comparative study of ^{19}F and ^{13}C is attempted since the factors which are important in determining the σ of ^{19}F are likely to be significant in the ^{13}C σ.[4]

Theory

The Hamiltonian of a molecule, containing a nuclear moment $\tilde{\mu}_A$, in a uniform magnetic field $\tilde{B}$ and an electric field $\tilde{F}$ is given (in a.u.) by[1]:

$$H(\tilde{\mu}, \tilde{B}, \tilde{F}) = H^{(000)} + H^{(100)}_{A\alpha}\,\mu_{A\alpha} + H^{(010)}_{\alpha}B_\alpha + H^{(001)}_{\alpha}F_\alpha + H^{(020)}_{\alpha\beta}\,B_\alpha B_\beta + H^{(110)}_{A\alpha\beta}\,\mu_{A\alpha}B_\beta + \ldots \quad (1)$$

with

$$H^{(100)}_{A\alpha} = \frac{-i}{c^2}\sum_j (r_{jA} \times \nabla_j)_\alpha / r^3_{jA}$$

$$H^{(010)}_{\alpha} = \frac{-i}{2}\sum_j (r_j \times \nabla_j)_\alpha$$

$$H^{(001)}_{\alpha} = \sum_j r_{j\alpha}$$

$$H^{(020)}_{\alpha\beta} = \frac{1}{8}\sum_j (r_j\,\delta_{\alpha\beta} - r_{ja}\,r_{j\beta})$$

$$H^{(110)}_{A\alpha\beta} = \frac{1}{2c^2}\sum_j (r_j \cdot r_{jA}\,\delta_{\alpha\beta} - r_{ja}\,r_{jA\beta}) / r^3_{jA}$$

where $\delta_{\alpha\beta}$ is the Kronecker delta and α, β are either x, y or z. A repeated Greek subscript indicates a summation over the three Cartesian Components.

In general the energy of a molecule in the above fields is given by[5]:

$$E(\tilde{\mu}, \tilde{B}, \tilde{F}) = E^{(000)} - P_\alpha F_\alpha - \frac{1}{2}\alpha_{\alpha\beta}F_\alpha F_\beta - \frac{1}{2}X_{\alpha\beta}B_\alpha B_\beta + \sigma_{\alpha\beta}\mu_{A\alpha}B_\beta \quad (2)$$

where P_α, $\alpha_{\alpha\beta}$, $X_{\alpha\beta}$ and $\sigma_{\alpha\beta}$ denote components of the permanent dipole moment, the polarisability, the magnetic susceptibility and the nuclear screening constant respectively.

So

$$\alpha_{\alpha\beta} = -\left(\frac{\partial^2 E}{\partial F_\alpha \partial F_\beta}\right)_0 \quad \text{and} \sigma_{\alpha\beta} = \left(\frac{\partial^2 E}{\partial \mu_{A\alpha}\, \partial B_\beta}\right)_0 \tag{3}$$

The subscript 0 implies that the derivatines are evaluated at $F_\alpha = F_\beta = \mu_{A\alpha} = B_\beta = 0$.

For a closed shell molecule described by a single determinant wave function the energy is given by[1,6]:

$$E = 2\mathrm{tr}\tilde{R}\tilde{f} + \mathrm{tr}\tilde{R}\tilde{G}(\tilde{R}) \tag{4}$$

where:
$\tilde{R}$: the density matrix,
$\tilde{f}$: the framework Hamiltonian and
$\tilde{G}(\tilde{R})$: the matrix which describes the electronic interactions.

Taking into account(1) $\tilde{f}$ can be expended as follows[5]:

$$\tilde{f} = \tilde{f}^{(000)} - i\tilde{f}^{(100)}\mu_{A\alpha} + i\tilde{f}_\alpha^{(010)} B_\alpha + \tilde{f}_\alpha^{(001)} F_\alpha - \tilde{f}_{\alpha\beta}^{(020)} B_\alpha B_\beta + \tilde{f}_{\alpha\beta}^{(110)} \mu_{A\alpha} B_\beta \tag{5}$$

A similar expansion holds for $\tilde{R}$.

On substituting the expanded $\tilde{f}$ and $\tilde{R}$ into (4) and differentiating with respect of F_α, F_β and $\mu_{A\alpha}$, B_β the $\alpha\alpha\beta$ and $\sigma_{\alpha\beta}$ result respectively:

$$\alpha_{\alpha\beta} = -2\mathrm{tr}\,\tilde{R}_\alpha^{(001)}\,\tilde{f}_\beta^{(001)} \tag{6}$$

and

$$\sigma_{\alpha\beta} = 2\mathrm{tr}\,[\tilde{f}_{\alpha\beta}^{(110)}\,\tilde{R}^{(000)} + \tilde{f}_\alpha^{(100)}\,\tilde{R}_\beta^{(010)}], \tag{7}$$

where $\tilde{R}_\alpha^{(001)}$ is the first order correction to the density matrix when it is perturbed by F_α and $\tilde{R}_\beta^{(10)}$ by B_β.[1,6]
Calculation of the matrices $\tilde{f}_\beta^{(001)}$, $\tilde{f}_{\alpha\beta}^{(110)}$ and $\tilde{f}_\alpha^{(100)}$ presents of course no problem. For the calculation of $\tilde{R}_\alpha^{(001)}$ — and similarly $\tilde{R}_\alpha^{(010)}$ — we will make use of the identity[1]:

$$\begin{aligned}\tilde{R}_\alpha^{(001)} &= \tilde{R}_0\tilde{R}_\alpha^{(001)}\tilde{R}_0 + \tilde{R}_0\tilde{R}_\alpha^{(001)}\tilde{R}'_0 + \tilde{R}'_0\tilde{R}_\alpha^{(001)}\tilde{R}_0 + \tilde{R}'_0\tilde{R}_\alpha^{(001)}\tilde{R}'_0 \\ &= \tilde{R}_{\alpha 11}^{(001)} + \tilde{R}_{\alpha 12}^{(001)} + \tilde{R}_{\alpha 21}^{(001)} + \tilde{R}_{\alpha 22}^{(001)}\end{aligned}$$

where $\tilde{R}_0 + \tilde{R}'_0 = \tilde{1}$

and $\tilde{R}_0 = \sum_{i(occ)} \tilde{C}_i\,\tilde{C}_i$, $\tilde{R}'_0 = \sum_{j(unocc)} \tilde{C}_j\,\tilde{C}_j$

namely $\tilde{C}_i$ and $\tilde{C}_j$ are the coefficients of the unperturbed occupied and unoccupied M.O.'s respectively.
On the other hand[1]

$$\tilde{R}_{\alpha 11}^{(001)} = \tilde{R}_{\alpha 22}^{(001)} = 0$$

and $$\tilde{R}_\alpha^{(001)} = \tilde{R}_{\alpha 12}^{(001)} + R_{\alpha 12}^{(001)\dagger}$$

So for the calculation of $\tilde{R}_\alpha^{(001)}$ we only need $\tilde{R}_{\alpha 12}^{(001)}$ which is iteratively found from:

$$\tilde{R}_{\alpha 12}^{(001)} = \sum_{\substack{i(occ) \\ j(unocc)}}' \frac{\Delta_{\alpha ij}^{(001)}}{E_i - E_j} \tilde{C}_i \tilde{C}_j\dagger$$

with $\Delta_{\alpha ij}^{(001)} = \tilde{C}_i^+ [\tilde{f}_\alpha^{(001)} + \tilde{G}(\tilde{R}_\alpha^{(001)})] \tilde{C}_j$

where E_k is the k_{th} unperturbed eigenvalue.
The average value, $<q>$, and the anisotropy, Δq, of a property q are determined by:

$$<q> = \frac{1}{3} q_{\alpha\alpha} \tag{8.1}$$

$$\Delta q = q_{zz} - \frac{1}{2}(q_{xx} + q_{yy}) \tag{8.2}$$

where $q_{\alpha\alpha}$ are the principal components of the property. For molecules with threefold or higher symmetry

$$\Delta_\alpha = \alpha_{11} - \alpha_i \tag{8.3}$$

where α_{11} is the polarisability along the axis of highest symmetry and α_i the polarisability perpendicular to this axis.

The nuclear screening constant can be resolved into diamagnetic and paramagnetic contributions, denoted by d and p respectively.

$$\sigma_{\alpha\beta} = \sigma_{\alpha\beta}^d + \sigma_{\alpha\beta}^p \tag{8.4}$$

Results and Discussion

For the calculation of $\alpha_{\alpha\beta}$ and $\sigma_{\alpha\beta}$ a minimal basis set has been used in which each STO (Slater type orbital) was simulated by 3 GTOs (Gaussian type orbitals). The exponents and the coefficients for this expansion were taken from Stewart[7] and the Slater exponents from Hehre et al.[8] The geometries of C_6H_6 1, 3, 5-$C_6H_3F_3$ and C_6F_6 were taken from the literature.[9] The origin of the coordinate system was taken at the centre of mass of the molecule. The ATMOL programme[10] as implemented on the Cambridge IMB 370/165 computer was used for the calculation of the SCF integrals, eigenvectors and eigenvalues.

a. The Polarisability

It is known that for all aromatic hydrocarbons $\Delta\alpha<0$[11]. This trend is followed by 1, 3, 5 - $C_6H_3F_3$ and C_6F_6 as well (Table I). This means that the

TABLE I

Molecule	$\alpha_{\perp} \times 10^{24}$, cm^3 calc.	$\alpha_{\parallel} \times 10^{24}$, cm^3 calc.	$\Delta\alpha \times 10^{24}$, cm^3 calc.	exp.[13]
C_6H_6	6.79 6.14[2]	0.87	−5.91	-5.59 ± 0.01
1, 3, 5-$C_6H_3F_3$	7.18	0.84	−6.33	-5.94 ± 0.05
C_6F_6	7.64	0.88	−6.76	-6.37 ± 0.05

$1\text{cm}^3 \approx 1.112650 \times 10^{-16}$ $\text{J}^{-1}\text{C}^2\text{M}^2$ (2)

electric field has the largest effect on the molecule when it is perpendicular to the highest axis of molecular symmetry.

The experimental polarisability anisotropies which are quoted in this work (Table I) have been measured using the Cotton-Mouton effect[12] according to which fluids show magnetic birefringence when a plane polarised light passes through them in a direction perpendicular to a strong magnetic field. In the vapour phase for axially symmetric molecules.[13]

$$ {}_{m}C = \frac{2}{135}\pi N\left[\eta + \frac{2}{3KT}\Delta\alpha\Delta\chi\right] \tag{9} $$

where:
${}_{m}C$: Cotton-Mouton constant,
N: Avogadro's number,
η: describes the effect of the magnetic field on the polarisability, and
$\Delta\chi$: the magnetic susceptibility anisotropy. Since the experiment was conducted in the vapour phase it is suitable for comparison with the present calculations performed on isolated molecules.

If $\Delta\chi \neq 0$ the first term in Eq. 9 is small and it can be neglected. Therefore from ${}_{m}C$ and $\Delta\alpha$ one can calculate $\Delta\chi$ which can be used to estimate the part of the NMR solvent shift which is due to the magnetic anisotropy of the solvent molecules[13].

From Table I one observes that $\alpha_{\perp}$ uniformely increases from C_6H_6 to C_6F_6 but $\alpha_{\parallel}$ presents a minimum at 1, 3, 5 - $C_6H_3F_3$. In addition C_6H_6 and C_6F_6 have approximately equal $\alpha_{\parallel}$. It is noteworthy that $|\Delta\alpha|$ slowly increases while $|\Delta\chi|$ rapidly decreases.[13] On the whole, it is seen from Table I, that the experimental and calculated polarisability anisotropies agree quite well. Finally from $\Delta\alpha$ and $\langle\alpha\rangle$ (see Eqs. 8 and Table I) one can calculate the depolarization ratio, p_0, of Rayleigh light scattered from the vapours of the examined compounds.[12]

$$P_0 = \frac{3d}{4d + 5} \quad \text{where} \quad d = \left(\frac{\Delta\alpha}{3 <\alpha>}\right)^2$$

So P_0 is 0.088, 0.0922 and 0.092 for C_6H_6, 1, 3, 5-$C_6H_3F_3$ and C_6F_6 respectively.

b. The nuclear screening constant

The present calculations show that (Table II), in agreement with well known experimental findings,[14] the ^{19}F nuclear screening constant is greatly influenced by a substituent at the ortho position. Both experiment and theory show that $<\sigma>$ and $\Delta\sigma$ of ^{19}F increase from 1, 3, 5-$C_6H_3F_3$ to C_6F_6.[15] $<\sigma>$ and $\Delta\sigma$ of ^{13}C increase from C_6H_6 to C_6F_6 (Table III). As one could expect the $\Delta\sigma$ of ^{13}C is larger when ^{13}C is bonded to ^{19}F than to ^{1}H. In addition the results of Table II support the assumption of Nehring and Saupe[16] that the diamagnetic part does not contribute significantly to fluorine $\Delta\sigma$. It is noteworthy that $\Delta\sigma^d$ contributes considerably to $\Delta\sigma$ of ^{13}C.

Snyder and Anderson[17] have found that the fluorine nuclei of C_6F_6 are most diamagnetically shielded when the applied field is perpendicular to the molecular plane. This was contrary to the conlusion of Andrew and Tunstall[18] based on their implementation of the semi-empirical theory of Karplus and Das.[19] The present calculations confirm the thesis of Snyder and Anderson (Table II). In 1, 3, 5-$C_6H_3F_3$ also, the most shielded component of ^{19}F is that along the axis perpendicular to the molecular plane. Apparently the most shielded element in a lot of fluorobenzenes is perpendicular to the molecular plane.[20] Further, according to the calculations which are in agreement with the experiment[3] in the aromatic carbons the most shielded component is that along the Z direction. The in plane shielding for the examined compounds is smaller suggesting that the planar ring effectively prevents the induction of a substantial secondary field at the corners of the ring.

Περίληψη

Ἡ Ἀνισοτροπία τῆς πολωσιμότητος καὶ τῆς σταθερᾶς πυρηνικῆς ἐπικαλύψεως τοῦ ^{13}C καὶ τοῦ ^{19}F τῶν C_6H_6, 1,3, 5-$C_6H_3F_3$ καὶ C_6F_6.

Ἡ θεωρία τῶν διαταραχῶν τοῦ McWeeny χρησιμοποιήθηκε γιὰ τὸν ὑπολογισμὸ τῆς πολωσιμότητος καὶ τῆς σταθερᾶς πυρηνικῆς ἐπικαλύψεως τοῦ ^{13}C καὶ τοῦ ^{19}F τῶν C_6H_6, 1, 3, 5-$C_6H_3F_3$ καὶ C_6F_6. Ἡ θεωρία τοῦ McWeeny ἔχει τὸ πλεονέκτημα ὅτι διαταράσσει τὴν μήτρα πυκνότητος μὲ ἀποτέλεσμα οἱ ὑπολογισμοὶ α) νὰ γίνονται σχετικὰ εὔκολα καὶ β) νὰ δίνουν ἀποτελέσματα ποὺ νὰ μποροῦν νὰ ἑρμηνευθοῦν κατὰ τρόπο χρήσιμο γιὰ τὸν χημικό.

Οἱ ὑπολογισμοὶ ἀπέδωσαν ἱκανοποιητικὰ τὰ κύρια χαρακτηριστικὰ τῶν πολωσιμοτήτων ποὺ ἐξετάστηκαν.

Οἱ παραπομπὲς 1 καὶ 3 εἶναι ἰδιαίτερα χρήσιμες γι' αὐτοὺς ποὺ ἐπιθυμοῦν περισσότερες λεπτομέρειες.

TABLE II. *^{19}F magnetic screening constants in ppm*

Molecule	σ^{d}_{xx}	σ^{d}_{yy}	σ^{d}_{zz}	σ^{p}_{xx}	σ^{p}_{yy}	σ^{p}_{zz}	σ_{xx}	σ_{yy}	σ_{zz}	$\Delta\sigma^{d}$	$\Delta\sigma^{p}$	$\Delta\sigma$	$\langle\sigma\rangle$
1. 3. 5-$C_6H_3F_3$	443.4	533.2	487.2	−155.2	−153.2	78.5	287.6	380.0	565.7	−1.1	233.0	231.9	411.1
C_6F_6	434.5	596.5	539.3	−106.1	−142.4	66.5	328.4	454.0	605.8	23.8	190.8	214.6	462.7

TABLE III. *^{13}C magnetic screening constants in ppm*

Molecule	σ^{d}_{xx}	σ^{d}_{yy}	σ^{d}_{zz}	σ^{p}_{xx}	σ^{p}_{yy}	σ^{p}_{zz}	σ_{xx}	σ_{yy}	σ_{zz}	$\Delta\sigma^{d}$	$\Delta\sigma^{p}$	$\Delta\sigma$	$\langle\sigma\rangle$
C_6H_6	304.7	387.8	404.3	−240.0	−281.3	−143.8	64.6	106.4	206.5	58.0	116.9	174.8	143.8
												180 ± 5[15]	
	a)												
1. 3. 5-$C_6H_3F_3$	484.6	420.4	611.0	−211.8	−277.2	−147.3	272.8	141.3	463.7	158.5	97.2	265.7	292.6
	b)												
	443.5	365.4	515.7	−249.2	−241.3	−147.7	194.3	124.3	367.9	113.3	97.6	208.9	228.8
C_6F_6	506.6	514.9	722.4	−222.2	−253.0	−146.3	284.4	261.8	576.0	211.6	91.3	302.9	374.0

a. Carbon bonded to fluorine
b. Carbon bonded to hydrogen

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Running title

Anisotropies of electric and magnetic properties.