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AN ESR INVESTIGATION OF SIDE-CHAIN OXIDATION AND HYDROGEN ABSTRACTION OF SOME PHENYL-SUBSTITUTED ALCOHOLS AND PHENYL-SUBSTITUTED ETHERS

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SUMMARY

E.s.r. spectroscopy has been employed to characterize radicals formed by the reaction of some phenyl-substituted alcohols and phenyl-substituted ethers with the hydroxyl radical HO• (from Ti^{3+}–H_2O_2 in a flow system) in aqueous solution, and with ^tBuO• (in situ photolytic decomposition of di-t-butyl peroxide) in benzene. In the first case, addition of HO• to the aromatic ring is followed at low pH by loss of OH^- and a benzylic proton or side-chain fragmentation. In the second case, for phenyl-substituted alcohols hydrogen abstraction by ^tBuO• represents the predominant route, and subsequent fragmentation occurs for some substrates. For phenyl-substituted ethers, benzylic hydrogen abstraction is predominant.

Key works: Electron spin resonance, benzyl radicals, phenyl-substituted alcohols and ethers, hydrogen abstraction, side-chain oxidation

INTRODUCTION

It has been shown that the hydroxyl radical (HO•) reacts (pH 3.5 — 9.5) with phenyl-substituted substrates to give hydroxycyclohexadienyl adducts[1]. As the pH is lowered, arene radical cations are formed which experimental evidence suggests are precursors for subsequent loss of proton of side-chain oxidation[2].

In the case of phenyl-substituted carboxylic acids, arene radical-cation formed at lower pH undergo proton-loss (to give benzylic radicals) or electron-transfer from the carboxyl group, followed by decarboxylation[3]. In the case of phenyl-substituted alcohols proton-loss, C_α—C_β bond scission and longer-range fragmentation have been observed under similar conditions[4].

The reaction of tert-butoxyl radical ^tBuO•, with hydrocarbon substrates in solution has been the subject of extensive

investigation.[5] $^{t}BuO\bullet$ readily abstracts a hydrogen atom from organic substrates to yield $^{t}BuOH$ and carbon-centered radicals which may be detected under experimental conditions or which may subsequently fragment. Radicals obtained from various ethers have been studied by e.s.r.[6].

We describe here the results of an investigation of radicals derived from phenyl-substituted alcohols and ethers by e.s.r. spectroscopy. We employed the Ti^{3+}—H_2O_2 flow system in aqueous solution and the in situ photolytic decomposition of di-t—butyl peroxide in benzene, in an attempt to compare the structural and electronic factors which govern the hydrogen loss and side-chain fragmentation.

EXPERIMENTAL

E.s.r. spectra were recorded with a Bruker ESP-300 spectrometer equipped with X-band Klystron and 100 KHz modulation. The hyperfine splitting and g-values were determined directly from the spectrometer's field scan. Splitting constants were measured to within ± 000.5 mT and g factors to within ± 0.0001 by comparison with aqueous solution of Fremy's salt, a (N)=1,3091 mT, g=2.0055.

In experiments with $HO\bullet$ the composition of the solutions were as follows: i) stream (1) contained Titanium (III) chloride (0.008 mol dm^{-3}), ii) stream (2) contained H_2O_2 (0.05 mol dm^{-3}), and iii) stream (3) contained the organic substrate (0.05-0.1 mol dm^{-3}).

In cases of low solubility in water, the substrate was divided into portions and added to the two other streams.

All solutions were degassed both before and during measurments by purging with oxygen-free nitrogen. The flow was maintained with a Watson-Marlowe 502 peristatic pump and a mixing chamber employed which allowed simultaneous mixing of the three reagent streams ca. 30 ms before passage through the cavity of the spectrometer. pH measurements were made using a Pye-Unican PW 9410 pH mater with the electrode into the

effluent stream. For experiments at pH less than 2.5 the desired amount of concentrated sulphuric acid was added to stream (1).

Photolysis was carred out on static samples with the unfiltered radiation from a Hanovia 977-I 1KW mercury — xenon compact arc. Solutions in benzene contained di-t-butyl peroxide (5% vol) and 10% vol of the substrate. Temperature was maintained at ca. 0 °C and was measured with the Bruker ER-4111 attachment. Solutions were deoxygenated for five minutes with oxygen-free nitrogen before photolysis.

Spectral simulations were carried out using a program (kindly provided by Dr.M.F.Chiu and modified to run on a VAX mainframe computer or on a BBC microcomputer) in which exchange and second-order effects were incorportated.

All phenyl-substituted alcohols were commercial samples (Aldrich) and were used as supplied.

Phenyl-substituted ethers were prepared from their equivalent phenyl-substituted alcohols. Alcohols were refluxed with sufficient amount of sodium metal in benzene for 15-17 hours until all traces of sodium were used up. Dimenthyl of diethyl sulfate (alkylating agents) were added and refluxing continued for another 8 hours. The product was washed first with NH_4OH and then separated by steam distillation. The mixture was washed 2-3 times with water and dried with $CaCl_2$. Benzene was removed by distillation and then the ethers were distilled under reduced pressure.[7] all ethers were tested for purity by standard spectroscopic methods (i.r., ^1H n.m.r. and m.s.).

TABLE I: Phenyl-substituted ethers prepared from the equivalent phenyl-substituted alcohols.

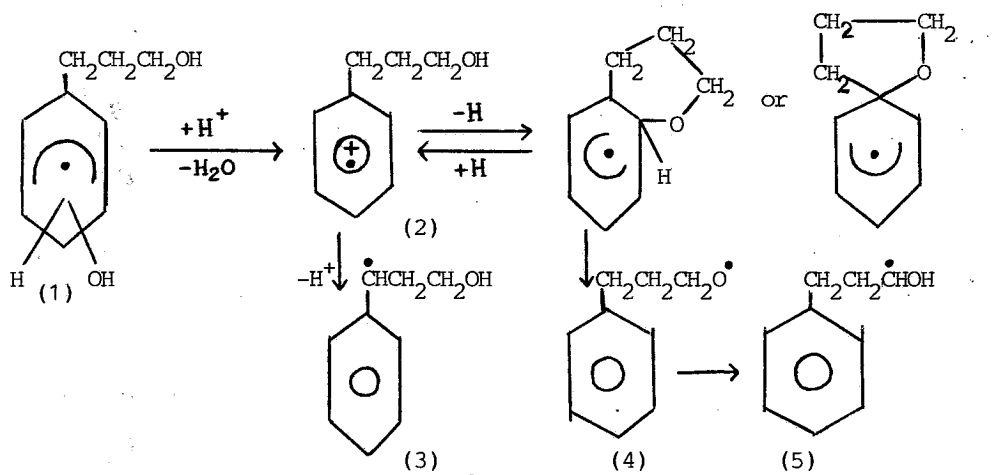
$PhCH_2-OCH_3$	85	169-172*
$PhCH_2-OCH_2CH_3$	78	185-187*
$PhCH(CH_3)-OCH_2CH_3$	83	182-184
$PhCH_2C(CH_3)_2CH_2-OCH_3$	67	218-220
$PhCH_2CH_2CH_2-OCH_3$	83	210-216
$PhC(CH_3)_2-OCH_3$	65	162-164
$PhCH_2CH_2-OCH_3$	85	192-194
$PhCH(CH_3)CH_2-OCH_3$	75	202-204
$PhCH_2CH_2C(CH_3)_2-OCH_3$	60	206-208
$PhCH(OCH_3)CH_2-OCH_3$	73	218-220

(* b.p.similar from reference 7)

RESULTS AND DISCUSSION

Radicals observed from the reaction of phenyl-substituted alcohols and ethers with HO•

In the pH range 2.5-9.5 all substrates studied gave complex spectra by their reaction with HO•, consistent with the formation of mixtures of hydroxycyclohexadienyl adducts (1). As the pH was lowered, new lines appeared due to radicals produced by two competing processess, namely oxidation at the side-chain terminus and loss of a benzylic hydrogen atom. The mechanism of this reaction may involve a radical-cation (2) (see Scheme 1) which can undergo deprotonation to give (3) or a competing internal nucleophilic attack to give (4) and ultimately (5) via a 1,2-H-shift.



Scheme 1

For example, benzyl alcohol gave $Ph\dot{C}HOH$ below pH ca. 2,5, this being the only radical observed below pH 1.5 (Table II), whereas in the case of 2-Phenylethanol fragmentation of the side-chain takes place at low pH (below pH ca. 2.2) giving the benzyl radical ($PhCH_2^{\bullet}$) as previously reported.[1,4] The equivalent methyl and ethyl ethers of these alcohols displayed only the loss of benzyl hydrogen. The formation of benzyl radicals of phenyl-substituted ethers indicates, as expected[8], that the ether function in the side-chain prevents the formation of the intermediate bicyclic radical and promotes the loss of a benzylic hydrogen in acidic conditions (Scheme 1).[8]

In the case of $PhCH(OH)CH_2OH$ at low pH the radicals delected were the $Ph\dot{C}(OH)CH_2OH$ due to the loss of a benzylic hydrogen and $Ph\dot{C}HOH$ which is formed by fragmentation and the production of CH_2O and H^+. The appropriate ether gave only the benzylic radical, $Ph\dot{C}(OCH_3)CH_2OCH_3$. Similar results were obtained with $PhCH_2CH_2CH_2OH$ which gave under acidic conditions the radicals $Ph\dot{C}HCH_2CH_2OH$ and $PhCH_2CH_2\dot{C}HOH$, but the benzylic radical for the corresponding ethers.

The radicals generated from $PhCH(OH_3)OH$ and $PhC(OH_3)_2OH$ at low pH (less than 2) gave the radicals $Ph\dot{C}(CH_3)OH$ and $\dot{CH_3}$

respectively. In the second case, fragmentation competes effectively with deprotonation when one or both fragments is relative stable[4].

$$[PhC(CH_3)_2OH]^{\bullet+} \longrightarrow PhC^+(OH)CH_3 + CH_3 \quad (1)$$

The corresponding ethers gave radicals with the loss of the benzylic hydrogen and the fragmentation of the C–O bond.

$$[PhC(CH_3)_2OCH_3]^{\bullet+} \longrightarrow PhC(CH_3)_2\dot{O}CH_2 + H^+ \longrightarrow Ph\dot{C}(CH_3)_2 + CH_2O \quad (2)$$

The radicals generated from two 3-(phenyl-substituted) alcohols and their appropriate ethers displayed novel fragmentation reactions. Thus $PhCH_2CH_2C(CH_3)_2OH$ gave $PhCH_2\dot{C}H_2$ below pH 4 and $Ph\dot{C}HCH_2C(CH_3)_2OH$ (the only signal detected below pH ca. 0.1). The second substrate, $PhCH_2C(CH_3)_2CH_2OH$, gave $PhCH_2\dot{C}(CH_3)_2$ and $PhCH_2C(CH_3)_2\dot{C}HOH$.

The appropriate methyl ethers gave only the benzylic radicals, $Ph\dot{C}HCH_2(CH_3)_2OCH_3$ and $Ph\dot{C}HC(CH_3)_2CH_2OCH_3$ respectively with the loss of a hydrogen atom.

The last two substrates of Table I, namely $PhCH(CH_2CH_3)CH_2OH$ and $PhCH_2OCH_2CH_2OH$ (benzyloxyethanol), gave radicals with the loss of a benzylic hydrogen atom. In the later case the benzylic radical is accompanied by a weaker signal assigned to $PhCH_2OCH_2\dot{C}HOH$.

Radicals observed from the reaction of phenyl-substituted alcohols and ethers with $^tBuO^\bullet$ in benzene.

Benzyl methyl ether and benzyl ethyl ether are the simplest phenylsubstituted ether which were studied in benzene (Table III)[6]. The radicals detected were: $PH\dot{C}HOCH_3$ and a mixture or $Ph\dot{C}HOCH_2CH_3$ and $Ph\dot{C}H_2$ respectively. It has been shown that some a-alkoxy alkyl radicals undergo a C–O bond scission (β scission) to yield a carbonyl compound and a alkyl radical,[9] [reaction (3)]:

TABLE II. E. s. r. parameters of radicals detected during the oxidation of phenyl - substituted alcohols and ethers by HO•. [a]

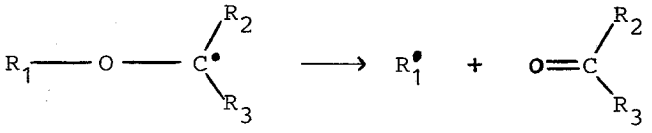
Substrate	Radical	Hyperifne splittings (mT)[b]						
		a(α-H)	a(β-H)	a(o-H)	a(m-H)	a(p-H)	a(other)	g[c]
$PhCH_2OH$	$PhC^{\bullet}HOH$	1.53	-	0.46 0.51	0.16	0.61	-	2.0031
$PhCH_2OCH_3$	$PhC^{\bullet}HOCH_3$	1.51	-	0.46	0.16	0.57	0.14 [d]	2.0031
$PhCH_2OCH_2CH_3$	$PhC^{\bullet}HOCH_2CH_3$	1.52	-	0.45 0.51	0.15 0.16	0.58	0.14 [e]	2.0031
$PhCH_2CH_2OH$	$PhCH_2^{\bullet}$	1.63	-	0.50	0.16	0.61	-	2.0025
$PhCH_2CH_2OCH_3$	$PhC^{\bullet}HCH_2OCH_3$	1.60	1.63	0.50	0.17	0.60	-	2.0026
$PhCH(OH)CH_2OH$	$PhC^{\bullet}(OH)CH_2OH$	-	1.60	0.50	0.16	0.60	-	2.0030
	$PhC^{\bullet}HOH$	1.53	-	0.46 0.52				2.0031
$PhCH(OCH_3)CH_2OCH_3$	$PhC^{\bullet}(OCH_3)CH_2OCH_3$	-	1.60	0.46	0.15	0.58	0.14 [d]	2.0031
$PhCH_2CH_2CH_2OH$	$PhC^{\bullet}HCH_2CH_2OH$	1.60	1.62	0.50	0.17	0.61	-	2.0026
	$PhCH_2CH_2C^{\bullet}HOH$	1.52	1.94	-	-	-	0.07 [f]	2.0030
$PhCH_2CH_2CH_2OCH_3$	$PhC^{\bullet}HCH_2CH_2OCH_3$	1.60	1.62	0.50	0.17	0.61	-	2.0026
$PhCH(CH_3)OH$	$PhC^{\bullet}(CH_3)OH$	-	1.60	0.50	0.15	0.60	-	2.0031
$PhCH(CH_3)OCH_2CH_3$	$PhC(CH_3)OCH_2CH_3$	-	1.45	0.50	0.16	0.60	-	2.0031
$PhC(CH_3)_2OH$	$CH_3^{\bullet}$	2.30	-	-	-	-	-	2.0025
$PhC(CH_3)_2OCH_3$	$PhC^{\bullet}(CH_3)_2$	-	1.60	0.50	0.16	0.60	-	2.0025
$PhCH_2CH_2(CH_3)_2OH$	$PhCH_2CH_2^{\bullet}$	2.18	2.77	-	-	-	-	2.0026
	$PhC^{\bullet}HCH_2C(CH_3)_2OH$	1.62	1.63	0.49	0.16	0.60	-	2.0026
$PhCH_2CH_2C(CH_3)_2OCH_3$	$PhC^{\bullet}HCH_2C(CH_3)_2OCH_3$	1.62	1.63	0.50	0.16	0.60	-	2.0026
$PhCH_2C(CH_3)_2CH_2OH$	$PhCH_2C^{\bullet}(CH_3)_2$	-	1.78 2.30	- (6H)	-	-	-	2.0026
	$PhCH_2C(CH_3)_2C^{\bullet}HOH$	1.53	-	-	-	-	0.07 [f]	2.0031
$PhCH_2C(CH_3)_2CH_2OCH_3$	$PhC^{\bullet}HC(CH_3)_2CHOCH_3$	1.60	-	0.50	0.16	0.60	-	2.0026
$PhCH(CH_2CH_3)CH_2OH$	$PhC^{\bullet}H(CH_2CH_3)CHOH$	1.53	1.84	-	-	-	-	2.0026
$PhCH_2OCH_2CH_2OH$	$PhC^{\bullet}HOCH_2CH_2OH$	1.52	-	0.50	0.16	0.60	0.14 [e]	2.0031
	$PhCH_2OCH_2C^{\bullet}HOH$	1.86	1.93	-	-	-	0.28 [f]	2.0030

[a] Hydroxycylohexadienyl radicals were detected from each substrate at higher pH. Data defer to acid conditions.
[b] ± 0.001 mT. [c] ± 0.0001. [d] $3H,OCH_3$. [e] $2H,OCH_2$. [f] 1H,OH

TABLE III. E. s. r. parameters of radicals detected in benzene solution by the in situ protolytic decomposition of di–tetr–butyl peroxide.

Substrate	Radical	Hyperifne splittings (mT)[a]						
		a(α-H)	a(β-H)	a(o-H)	a(m-H)	a(p-H)	a(other)	g[b]
$PhCH_2OCH_3$	$PhCHOCH_3$	1.51	-	0.4 0.51	0.16 0.15	0.57	0.14 [c]	2.0031
$PhCH_2OCH_2CH_3$	$PhCHOCH_2CH_3$	1.52	-	0.5	0.16	0.57	0.14 [d]	2.0031
	$PhCH_2$	1.63	-	0.5	0.17	0.61	-	2.0026
$PhCH_2CH_2OH$	$PhCH_2CHOH$	1.50	2.10	-	-	-	0.06 [e]	2.0031
$PhCH_2CH_2OCH_3$	$PhCH_2CH_2OCH_2$	1.65	-	-	-	-	0.19 [d]	2.0030
	$PhCH_2CHOCH_3$	1.39	1.96	-	-	-	0.15 [c]	2.0030
$PhCH(OH)CH_2OH$	$PhC(OH)CH_2OH$	-	1.60	0.46 0.51	0.16	0.60	0.06 [e]	2.0031
$PhCH(OCH_3)CH_2OCH_3$	$PhC(OCH_3)CH_2OCH_3$	-	1.63	0.46	0.16	0.60	0.14 [c]	2.0031
$PhCH_2CH_2CH_2OH$	$PhCH_2CH_2CHOH$	1.52	1.85	-	-	-	0.06 [e]	2.0030
	$PhCHCH_2CH_2OH$	1.53	1.63	0.50	0.16	0.60	-	2.0026
$PhCH_2CH_2CH_2OCH_3$	$PhCH_2CH_2CH_2OCH_2$	1.65	-	-	-	-	0.21 [d]	2.0034
	$PhCH_2CH_2CHOCH_3$	1.67	1.85	-	-	-	0.14 [c]	2.0035
$PhCH(CH_3)OH$	$PhC(CH_3)OH$	-	1.60	0.50	0.16	0.60	-	2.0031
$PhCH(CH_3)OCH_2CH_3$	$PhCH(CH_3)OCHCH_3$	1.40	2.15	-	-	-	-	2.0030
	$PhC(CH_3)OCH_2CH_3$	-	1.60	0.50	0.16	0.60	-	2.0026
$PhC(CH_3)_2OH$	CH_3	2.30	-	-	-	-	-	2.0025
$Ph(CH_3)_2OCH_3$	$PhC(CH_3)_2$	-	1.60	0.50	0.16	0.60	-	2.0031
	$PhC(CH_3)_2OCH_2$	1.70	-	-	-	-	-	2.0025
$PhCH_2CH_2C(CH_3)_2OH$	$PhCHCH_2C(CH_3)_2OH$	1.62	1.63	0.49	0.16	0.60	-	2.0026
$PhCH_2CH_2C(CH_3)_2OCH_3$	$PhCHCH_2C(CH_3)_2OCH_3$	1.62	1.63	0.50	0.16	0.60	-	2.0026
	$PhCH_2CH_2C(CH_3)_2$	-	1.63 1.60 (6H)	-	-	-	-	2.0026
$PhCH_2C(CH_3)_2CH_2OH$	$PhCH_2C(CH_3)_2CHOH$	1.53	-	-	-	-	0.07 [e]	2.0031
	$PhCH_2C(CH_3)_2$ (trace)	-	1.78 2.30 (6H)	-	-	-	-	2.0026
$PhCH_2C(CH_3)_2CH_2OCH_3$	$PhCH_2C(CH_3)_2CH_2OCH_2$	1.63	-	-	-	-	0.23 [d]	2.0031
	$PhCH_2C(CH_3)_2CHOCH_3$	1.35	-	-	-	-	0.14 [c]	2.0030

[a] ± 0.01 mT. [b] ± 0.0001. [c] 3H,OCH_3. [d] 2H,OCH_2. [e] 1H,OH



(3)

The 2-(phenyl-substituted) and 3-(phenyl-substituted) alcohols and their appropriate ethers, namely $PhCH_2CH_2OH$, $PhCH_2CH_2CH_2OH$, $PhCH_2CH_2OCH_3$ and $PhCH_2CH_2CH_2OCH_3$, gave radicals due to the loss of a hydrogen atom from the $-CH_2$ and $-CH_3$ groups adjacent to the oxygen atom. $PhCH_2CH_2CH_2OH$ with a the abstraction of one benzyl hydrogen gave $PhCHCH_2CH_2OH$.

Phenyl-substituted alcohols and their appropriate ethers with branched alkyl groups, $PhCH(CH_3)OH$, $PhCH(CH_3)OCH_2CH_3$ and $PhC(CH_3)_2OCH_3$, gave radicals due to the loss of a hydrogen atom, benzylic or adjacent to the oxygen. The signal of CH_3 from $PhC(CH_3)_2OH$ presumably reflects O-H abstraction followed by fragmentation reaction (4) :

$$PhC(CH_3)_2OH + {}^tBuO \longrightarrow {}^tBuOH + PhC(CH_3)_2O \longrightarrow PhCOCH_3 + CH_3 \quad (4)$$

The 3-(phenyl-substituted) alcohols and their appropriate ehters gave a mixture of radicals for each substrate. The radicals generated were the results of loss of a benzylic of etheric hydrogen, but fragmentation takes place also.

$$ {}^tBuO + PhCH_2C(CH_3)_2CH_2OH \longrightarrow {}^tBuOH + PhCH_2C(CH_3)_2CH_2O \longrightarrow$$
$$\longrightarrow PhCH_2C(CH_3)_2 + CH_2O \quad (5)$$

$$ {}^tBuO + PhCH_2CH_2C(CH_3)_2OCH_3 \longrightarrow {}^tBuOH + PhCh_2CH_2C(CH_3)_2OCH_2 \longrightarrow$$
$$\longrightarrow PhCH_2CH_2C(CH_3)_2 + CH_2O \quad (6)$$

CONCLUSIONS

From the results of the present study the following conclusions can be drawn.

1. Phenyl-substituted alcohols under acidic conditions (pH lower that 2) react with the hydroxyl radical (HO) in aqueous solution giving radicals which may be formed by the loss of a hydrogen atom or by fragmentation via radical-cations.

In contrast phenyl-substituted ethers give radicals with the loss of a benzylic hydrogen atom while fragmentation occurs in only a few cases (see Table II).

2. Phenyl-substituted alcohols react with $^{t}BuO^{\bullet}$ in benzene solution to give a mixture of radicals due to the loss of one hydrogen atom (usually a benzylic or from $-CH_2$ groups adjacent to the hydroxyl group). Fragmentation occurs in a few cases and only when one or both fragments is relatively stable. Probably via H-elimination from the OH-group which leads to an alkoxy radical and the subsequent loss of CH_2O. Phenyl-substituted ethers give radicals due to the loss of a hydrogen atom from $-CH_2$ or $-CH_3$ groups adjacent to the ether's oxygen followed in some cases by fragmentation.

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ΠΕΡΙΛΗΨΗ "Μελέτη ESR της οξείδωσης πλευρικής αλυσίδας και απόσπασης υδρογόνου από φαινυλο-υποκατεστημένες αλκοόλες και φαινυλο-υποκατεστημένους αιθέρες)

Η φασματοσκοπία ESR χρησιμοποιήθηκε για τον χαρακτηρισμό ελευθέρων ριζών που σχηματίσθηκαν από μερικές φαινυλο-υποκατεστημένες αλκοόλες και τους αντίστοιχους αιθέρες τους. Οι ελεύθερες ρίζες δημιουργήθηκαν με αντίδραση με ρίζα υδροξυλίου, HO (από το σύστημα $Ti^{3+}-H_2O_2$) σε υδατικά διαλύματα, και με την ρίζα $^{t}BuO^{\bullet}$ (από φωτοχημική διάσπαση του δι-t-βουτυλο-υπεροξειδίου) σε βενζόλιο. Στην πρώτη μέθοδο, η προσθήση της $HO^{\bullet}$ στον αρωματικό δακτύλιο συνοδεύεται σε χαμηλότερο pH με απόσταση υδρογόνου ή οξείδωση της πλευρικής αλυσίδας. Στη δεύτερη μέθοδο, οι φαινυλο-υποκατεστημένες αλκοόλες παρουσιάζουν απόσπαση υδρογόνου σαν την κύρια αντίδραση, ενώ η κατάτμηση δεσμών συμβαίνει σε μερικές περιπτώσεις. Οι φαινυλο-υποκατεστημένοι αιθέρες παρουσιάζουν κυρίως την απόσπαση βενζυλικού υδρογόνου.

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CATIONIC POLYMERISATION OF DIENES BY THE HOMOGENEOUS ZIEGLER-NATTA CATALYST $Cp_2TiCl_2/Et_3Al_2Cl_3$.

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SUMMARY

A series of dienes, conjugated and not conjugated, cyclic and open chain ones are polymerised by the Ziegler-Natta catalyst $Cp_2TiCl_2/Et_3Al_2Cl_3$. For the first time the cationic polymerisation of bicyclo-[2.2.1]-hepta-2,5-diene (norbornadiene-NBDE), 1,3-cyclooctadiene (COD), 1,3-cyclohexadiene (CHD), cis and trans-1,3-pentadiene (PD), 1,5-hexadiene (HD) and 1,7-octadiene (OD) by the above mentioned Ziegler-Natta catalyst is confirmed. The resultant polymers, especially the conjugated acyclic dienes, have no stereospecifity and the observed loss of double bond is attributed to the transannular, branched, cyclic and crosslinked products. The cationic character of the resultant polymers was confirmed by means of ^{1}H-NMR, IR, GPC and UV analyses.

Key words: Polymerization of Dienes/Ziegler-Natta Catalyst/Cationic polymerisation.

INTRODUCTION

Recent studies of Group IV metallocene-alkylaluminium catalytic systems show that these catalysts give active cationic species[1]. The cationic nature of titanocene based Ziegler-Natta catalysts was suggested by D'yachkovskii et al.[2] for $Cp_2TiCl_2/MeAlCl_2$ after electrochemical studies proposing $[Cp_2TiMe]^+$ as the active species. In agreement with the above suggestion cationic dicyclopentadienyl Zr(IV) complexes have been found to polymerise ethylene without an Al cocatalyst[3-5].

Also, the cationic species for the system $Cp_2HfCl_2/EtAlCl_2$[6] and the species produced by reaction between Cp_2ZrCl_2 and methylalumoxane[7] have been postulated. In a earlier publication we presented the results of experiments performed in our laboratory concerning the catalytic systems of metallocene-dichlorides (M=Ti, Hf, Zr) with different alkylaluminiums on the cationic polymerisation of 1,5-cyclooctadiene[8] and phenyl-acetylene[9].

In this paper the polymerisation of a series of dienes, conjugated and not conjugated, cyclic and acyclic ones by the Ziegler-Natta catalyst $Cp_2TiCl_2/Et_3Al_2Cl_3$ is reported for the first time. The polymerised dienes are: bicyclo-[2.2.1]-hepta-2,5-diene (norbornadiene-NBDE), 1,3-cyclooctadiene (COD), 1,3-cyclohexadiene (CHD), cis and trans-1,3-pentadiene (PD), 1,5-hexadiene (HD) and 1,7-octadiene (OD).

Our results are compared with those obtained in the literature[10-32] for the cationic polymerisation of dienes and indicate that the catalyst $Cp_2TiCl_2/Et_3Al_2Cl_3$ is cationic.

EXPERIMENTAL

Materials: The monomers bicyclo-[2.2.1]-hepta-2,5-diene, 1,3-cyclooctadiene, 1,3-cyclohexadiene, cis and trans 1,3-pentadiene, 1,5-hexadiene and 1,7-octadiene (Fluka AG) were distilled at reduced pressure under argon from CaH_2 and were used immediately. $Et_3Al_2Cl_3$ (Aldrich Chemical Company) and Cp_2TiCl_2 (Fluka AG) were used as received. Dichloromethane was refluxed over CaH_2 for 24 h, distilled and stored under an argon atmosphere.

Polymerisation: The polymerisation reactions were conducted at room temperature in CH_2Cl_2 in a Schlenk tube under argon. The catalyst/cocatalyst mole ratio was 1/6. The catalyst (Cp_2TiCl_2) concentration was 10^{-2} M and the catalyst/monomer mole ratio was between 1/50 and 1/200. The ratios was found by monomer consumption (1,5-cyclooctadiene) of the system ($Cp_2TiCl_2/Et_3Al_2Cl_3$)[8]. The total volume of the reaction solution was 20 ml. During the polymerisation of the above mentioned monomers the following changes in the the colour of the reaction solution were observed: The coloured solution turns from red to brown with the addition of the cocatalyst followed by a further change to green and finally brown with the monomer addition. The monomer and the cocatalysts were added by hypodermic syringes. The end of reaction was determined by monomer consumption. Moreover consumption was established by monitoring its concentration by GC. The polymerisation was stopped by pouring the reaction mixture into methanol and

hydrochloric acid (10%). The precipitated white powder was collected, dissolved in a small amount of chloroform, and precipitated again as mentioned above. Finally the polymer generated was dried to constant weight in vacuo at 30°C for 24 h.

Measurements: GC data were obtained with a Perkin-Elmer 8310B instrument equipped with an OV-101 column and a flame ionization detector. GPC data were obtained using a Waters Associates 401 Liquid Chromatography apparatus equipped with a differential refractometer as a detector, using toluene as eluent with ultrastyragel (500, 10^2 A°) pore size columns in series. Polystyrene was used for calibration. Infra-red spectra were obtained with a Perkin-Elmer 783B spectrometer. The ^{1}H-NMR spectra were obtained in $CDCl_3$ using a Varian FT 80A spectrometer at room temperature. The chemical shifts were measured with respect to TMS as internal standard. The UV spectra were recorded using a Perkin Elmer, Model Lamda 15 spectrometer. UV studies were taken into 1cm cell under argon. Solutions of polymers (0,1% v/v) in hexane were added by syringe.

RESULTS AND DISCUSSION

The results of polymerisation of dienes initiated by the Ziegler-Natta catalyst Cp_2TiCl_2 /$Et_3Al_2Cl_3$ are summarised in Table I.

As it shown in Table I the resultant polymers are amorphous or crosslinked and no stereospecifity is observed. The incomplete monomer conversion observed is attributed to the reduction of the catalyst ($Ti^{IV} \rightarrow Ti^{III}$)[2,8].

In Table II the weight and number-average molecular weights of the polymers and PDI are given. The molecular weights are low because of the disappearance of the catalytic center (reduction $Ti^{IV} \rightarrow Ti^{III}$).

With respect to the characterization of the polymers; the polydispersity of polydienes, the low molecular weights, the monomodal molecular distribution of GPC curve (with the exception of poly-norbornadienes and poly-pentadienes) (Table II) and the polarity of the solvent indicate that the reaction of dienes is a cationic polymerisation in agreement with the spectroscopic data following below. For poly-norbornadiene and poly-pentadienes the molecular distribution is bimodal and is believed that cationic polymerisations present a bimodal distribution when the polymeric chains take place via two types of intermediates[33].

The high value of PDI of poly-norbornadiene is attributed to the resultant branched

Table I. Conditions and results of the polymerisation of dienes by the catalytic system Cp_2TiCl_2/Et_3AlCl_3.

Run No	Monomer	molar ratio cat./ monomer	Monomer (%) consumption	time*	Polymer yield %	
					Amorphus	Grosslinked
1	norbornadiene	1/200	100	30min	—	100
2	»	1/50	100	40min	65	28
3	1,3-cyclooctadiene	1/200	17	2 h	15	—
4	1,3-cyclohexadiene	1/200	100	3 h	82	—
5	cis-pentadiene	1/100	—	24 h**	62	—
6	trans-pentadiene	1/100	—	24 h**	82	—
7	1,5-hexadiene	1/100	75	1 h	40	—
8	1,7-octadiene	1/200	100	60min	—	97

$[Cp_2TiCl_2] = 10^{-2}M$, [Ti]/[Al]=1/6. Solvent dichloromethane. Temperature 25°C.

* time of monomer consumption

** duration of polymerisation

Table II: $\overline{M}_w$, $\overline{M}_n$ and polydispersities of poly-dienes.

Run No	Polymer	molar ratio cat./monomer	$\overline{M}_w$*	$\overline{M}_w$*	$I = \overline{M}/\overline{M}_n$
1	poly-norbornadiene**	1/200	—	—	—
2	»	1/50	31600	1545	20.00
3	poly-1,3-cyclooctadiene	1/200	4190	3290	1.27
4	poly-1,3-cyclohexadiene	1/200	5900	2320	2.54
5	poly-(cis-1,3-pentadiene)	1/100	3920	2900	1.35
6	poly-(trans-1,3-pentadiene)	1/100	3570	2970	1.20
7	poly-1,5-hexadiene	1/100	3300	1130	2.53
8	poly-1,7-octadiene**	1/200			—

* by GPC in toluene at 25°C

** crosslinked polymers

polymer. Among the side products the addition products of norbornadiene with Cl_2 and HCl have been found as it has been described already[34]. Moreover the Friedel-Crafts alkylation product of the solvent has been also reported elsewhere[35]. In this Ref.[35] the -CH_2Cl endgroups have their corresponding band on ^{1}H-NMR spectra at 3.41 ppm in complete agreement with our results.

Characterisation of the polymers

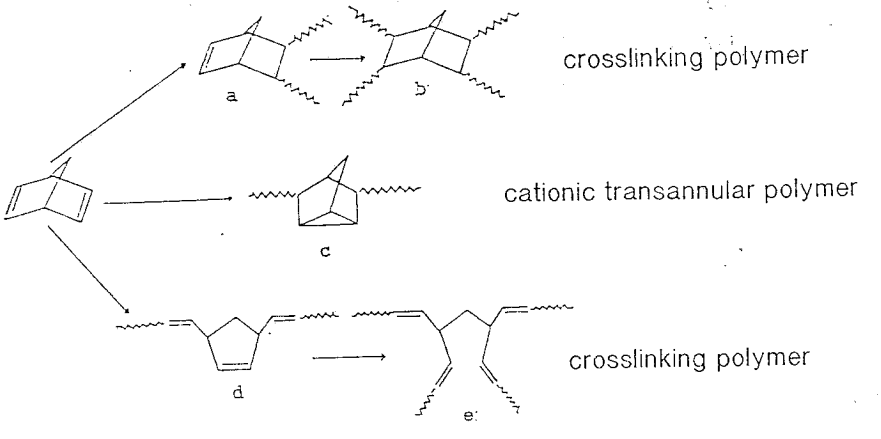
The poly-NBDE

As it is referred in the literature the polymerisation of bicyclic and not conjugated diene NBDE by cationic catalysts[10-14] resulted the cationic transannular polymer.

This monomer offers at least three possibilities for homopolymerisation as shown in Scheme I.

The third possibility is the ring opening polymerisation which is usually carried out by using Ziegler-Natta catalytic systems and these of metathesis polymerisation[36-38]. This possibility has to be excluded because there are no carbon-carbon double bonds as shown in IR and ^{1}H-NMR spectra.

The resultant poly-NBDE taken by Run 1 in Table I with a ratio of cat./monomer=1/200 is crosslinked because it is insoluble in any organic solvent. In the IR spectra there is an absorption band at 810cm^{-1} characterised by the repeating nortricyclene (c)[12,13] in Scheme I and another absorption band at 1300 cm^{-1} due to the presence of the bridging methylene group for the repeating unit[12,13]. Thus it appears that the crosslinked poly-NBDE incorporates both repeating units **b** and **c**.



Scheme I

The resultant poly-NBDE taken by Run 2 in Table II where cat./monomer=1/50 is amorphous. In the IR spectra (FIG. 1) there is a weak absorption band at 1620cm^{-1} due to the carbon-carbon double bond. This weak band conserning the absorption at 1300cm^{-1} [12,13] due to the bridging methylene group, belongs to the repeating unit **a** and not to the unit **d** because in the last unit the bridging methylene group does not exist. Also in the IR spectrum the absorption band at 810cm^{-1} [12,13] is due to the unit **c**. Thus this polymer incorporates both repeating units **a** and **c**.

The ^{1}H-NMR spectra of poly-NBDE confirms the structure indicated by the IR spectra. In the ^{1}H-NMR spectra there are chemical shift of cyclopropane at 0,9 ppm and the chemical shift of -CH_2 in a **a**-position to the cyclopropane ring at 1.35 ppm[39] are observed as well as the residual carbon-carbon double bonds at 5,8-6,2 ppm

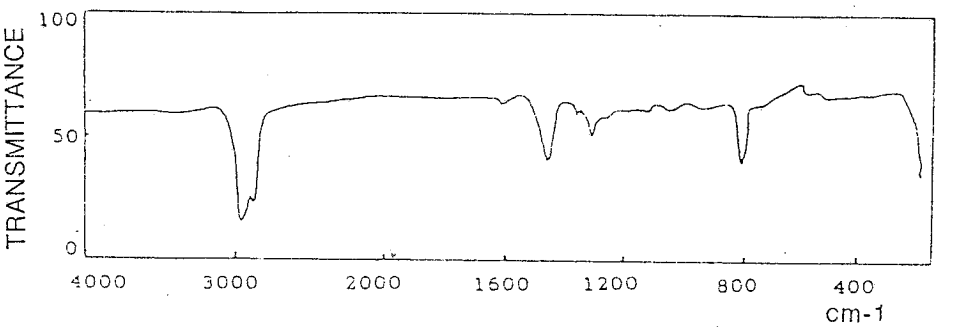
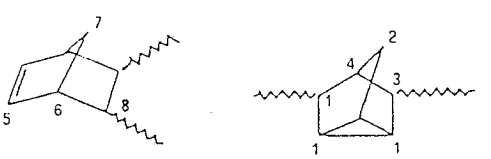


FIG 1. *IR spectra of poly-NBDE (amorphous)*

which characterise the unit **a**[12,13]. The chemical shifts of the carbon-carbon double bonds for the unit **d** are in the area 5,1-5,7ppm[38]. As it is shown in Scheme II the ratio $H_5/H_1+H_2+H_3+H_4+H_6+H_7+H_8$ indicating the ratio of olefinic protons to the total amount of the aliphatic protons of the polymer, is 1/7. From ^{1}H-NMR spectra integration the above ratio is calculated to be 1/63. So the percentage of the **a** unit is 11% and that of the **c** unit is 89%.

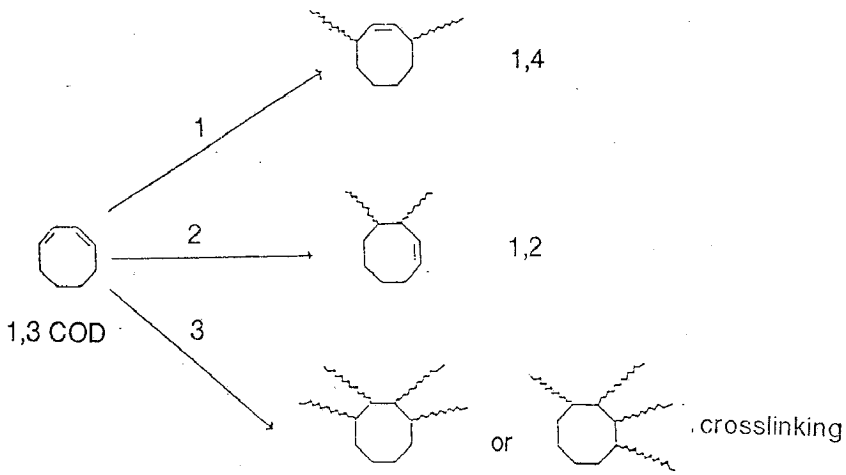


Scheme II

The poly-(1,3-COD).

The conjugated cyclic dienes (1,3-COD and 1,3-CHD) were polymerised by cationic catalysts[12-22] and the polymers obtained consisted of either 1,4 or 1,2 structural units. These polymers were oxidized by air and the loss of the double bond was attributed to the chain branching.

Three possible types of polymers (1,2-1,4 and crosslinked) may arise through the cationic polymerisation of 1,3-COD[15-17] as shown in Scheme III.



Scheme III

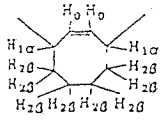
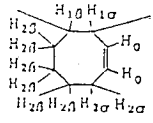
The resultant polymer is a white amorphous powder, soluble in organic solvents and so the third possibility of crosslinking polymer has to be excluded. In the IR spectra there is an absorption band at 1650cm^{-1} due to the carbon-carbon double bond and an absorption band at 1720cm^{-1} due to the carbonyl group (C=O) resulting from the oxidation of carbon-carbon double bond.

The ^{1}H-NMR spectrum of poly-(1,3-COD) exhibits broad resonances at 1.27ppm (H_{1b}) and 2.20ppm(H_{1a}) characterizing the methine protons and at 1.54ppm (H_{2b}) and 2.5ppm(H_{2a}) characterizing the methylene protons[6]. The olefinic protons (H_o) exhibit three resonances at 5.36ppm, 5.68ppm and 5.8ppm representing the chemical shifts of single double bond, the conjugated diene and conjugated triene[40] respectively.

The ratio $H_o/H_{1a}+H_{2b}+H_{1b}+H_{2a}$ (Table III) is a measure of the degree of branching or crosslinking that is present in the polymer. In the present case the value of the above ratio is 1/17 which means that the degree of branching or crosslinking is about 70%.

The ratio of allylic to the aliphatic protons is the only available integration on the ^{1}H-NMR spectrum for the determination of the 1,4 or the 1,2 structural uints. In the present case the value of the above ratio is 1/2.34 which means that the polymer obtained consists predominantly 1,2 structural units.

Table III. Expected ^{1}H-NMR data for poly-(1,3-cyclooctadiene)

STRUCTURE	 1,4	 1,2

$H_o/H_{1a} + H_{2b} + H_{1b} + H_{2a} = 1/5$

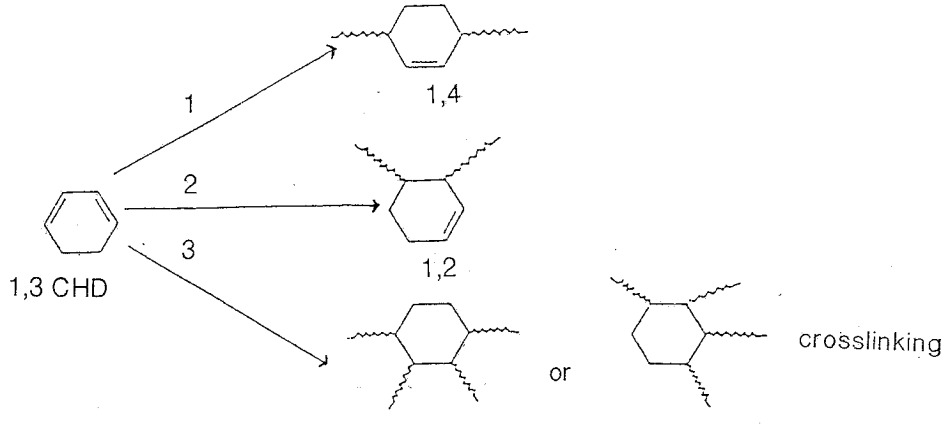
$H_{1a} + H_{2a}/H_{1b} + H_{2b} = 1/2.33$

The poly-(1,3-CHD)

This monomer offers three possibilities for cationic polymerisation[12-16,18-22] as shown in Scheme IV.

The resultant polymer is an amorphous white powder soluble in organic solvent and so the third possibility of crosslinked polymer has to be excluded.

In the IR spectrum of poly-(1,3-CHD) there is an absorption band at 1650cm^{-1} due to the carbon-carbon double bond and three absorption bands at 970cm^{-1}, 900cm^{-1} and 740cm^{-1} representing respectively the 1,4 trans, the 1,2 and the



Scheme IV

1,4-cis structural units. Thus the poly-(1,3-CHD) incorporates the structural units 1,4 and 1,2.

The ^{1}H-NMR spectra of poly-(1,3-CHD) (FIG. 2) exhibit the broad resonances of methine protons H_b at 1.27ppm of methylene protons H_b and H_a at 1.53ppm and 2.32ppm respectively, and of the olefinic protons H_o at 5.62 ppm[15,41] (Table IV).

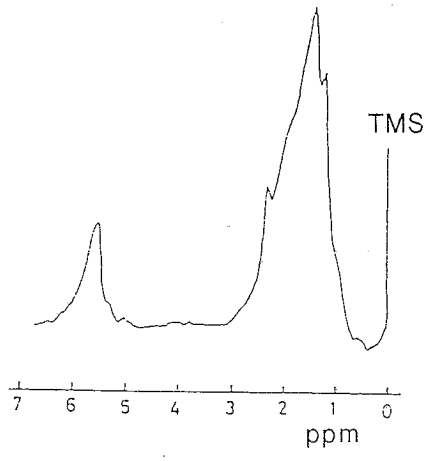


FIG 2. *^{1}H-NMR spectra of poly-(1,3-CHD)*

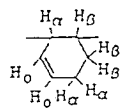
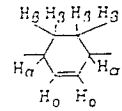
The ratio H_o/H_a+H_b (Table IV) represents the degree of branching or crosslinking of the polymer. In the present case the value of the above ratio is 1/5 which means that the degree of branching or crosslinking is 40%. Consequently the amount of 1,4 and 1,2 structural units is 60%.

The poly-(cis-1,3-pentadiene) and the poly-(trans-1,3-pentadiene).

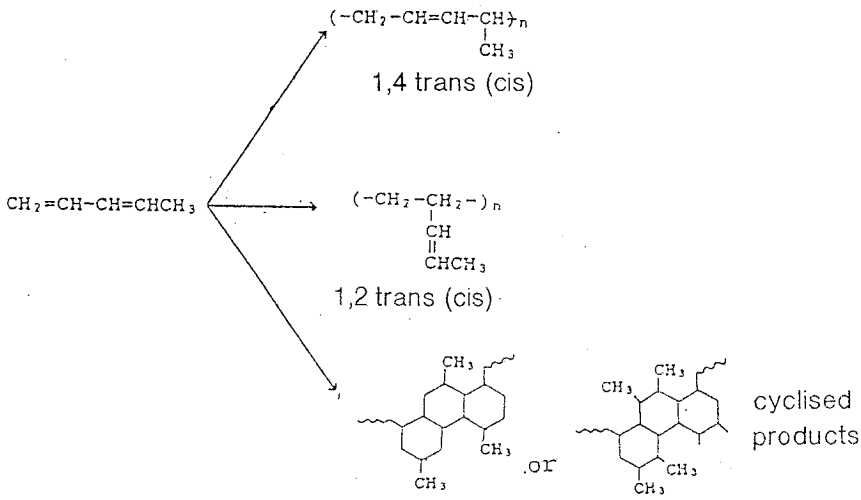
The acyclic conjugated dienes cis and trans-1,3-pentadienes were polymerised by Ziegler-Natta[23-27] and cationic catalysts[28,29]. With Ziegler-Natta catalysts was obtained stereoselective polymerisations and in the case of cationic catalysts the polymer obtained consisted of 1,4 and 1,2 structural units and a large extend of cyclic products.

The possible types of polymers (1,4-1,2 and cyclic structure) from the polymerisation of cis and trans-1,3-pentadiene with Ziegler-Natta and cationic catalysts are shown in Scheme V.

Table IV. Expected ^{1}H-NMR data for poly-(1,3-cyclohexadiene)

	1,2-poly-(1,3-cyclohexadiene)	1,4-poly-(1,3-cyclohexadiene)
STRUCTURE		
$H_o/H_a + H_b$	1/3	1/3

The resultant polymers are amorphous white powders. The absorption bands in the IR spectra of polypentadienes are shown in Table V.



Scheme V

The spectra of cis and trans-pentadiene show an absorption band at 1660cm^{-1} due to the carbon-carbon double bond and an absorption band at 965cm^{-1} due to both 1,4trans+1,2trans structures. The distinction between these structures becomes possible with the analysis of the absorption bands at 1375cm^{-1} and 1370cm^{-1} which are due to the 1,2-trans and 1,4-trans structures respectively. As shown in Table V there are no absorption bands for the 1,4 cis and 1,2 cis structure. So the polymers resulting from cis and trans 1,3-pentadiene consist of 1,4 trans and 1,2 trans structure only.

Table V. Absorption bands of polypentadienes IR spectra

	V, cm^{-1}		
	values of absorpiton cited in literature[16,20]	cis-1,3-pentadiene	trans-1,3-pentadiene
Vas CH_3	2960	2960	2960
Vas CH_2	2920	2920	2920
Vs CH_3	2880	2880	2860
Vs CH_2	2840	2840	—
V (C=C)	1660	1660	1660
δCH_2	1460	1460	1465
$\delta s\ CH_3$	1450	1450	1455
$\sigma_{as}\ CH_3$ (1,2 trans)	1375	1375	1375
$\sigma_{as}\ CH_3$ (1,4 trans)	1370	1370	1370
σ(CH=CH) (1,4 trans)	965	965	965
γ(CH=CH_2) (3,4)	910	—	—
γ(CH=CH) (1,4 cis)	760	—	—
γ(CH=CH) (1,2 cis)	730	—	—

The cyclization products that are formed (Scheme V) make the ^{1}H-NMR analysis of the polypentadienes difficult. The estimated ratio of the olefinic protons to the total amount of the aliphatic protons of the polypentadienes which were found by integration of the ^{1}H-NMR spectra, shows that the percentage of the crosslinking products is 68% for the cis-1,3-pentadiene and 70% for the trans-1,3-pentadiene. The 1,2 trans and 1,4 trans structure that was found in IR spectra was assigned in ^{1}H-NMR spectra at 1.24ppm and 1.95ppm respectively[41]. If in the case of poly-(1,3-pentadiene) we ignore the cyclised products, according to the Ref.[42] the percentage of 1,4-trans structure is 48% and for the 1,2-trans is 52%. Similarly the corresponding amounts for the poly-(trans-1,3-pentadiene) is 60% and 40%. The existence of the cyclised products makes the percentage of the 1,2 trans structure higher and that of the 1,4 trans structure lower. In Table VI the composition of the polypentadienes is presented.

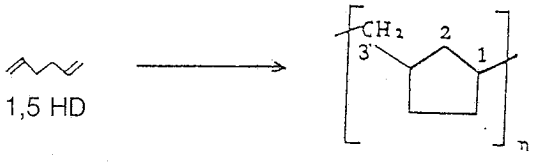
The poly-(1,5-hexadiene)

The α,ω acyclic diene 1,5-hexadiene and 1,7-octadiene with Ziegler-Natta catalyst[30-32] the resultant polymer was a cyclised product and a crosslinked product as a side reactions.

Table VI. Composition of polypentadienes

	cis-1,3-pentadiene	trans-1,3-pentadiene
cyclised products	68%	70%
trans 1,4	48%	60%
trans 1,2	52%	40%

This monomer offers one possibility for Ziegler-Natta polymerisation[30,31] as shown in Scheme VI.



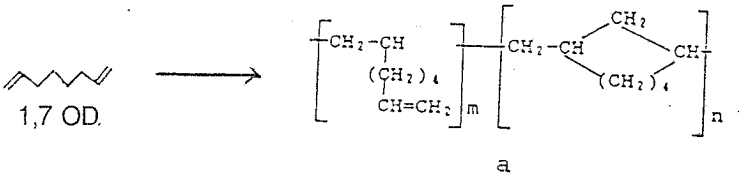
a

Scheme VI

The IR spectra of poly-1,5-hexadiene shows the methylene and methyl absorbance at 2960, 2840, 1465 and 1450cm^{-1}. Also in the ^{1}H-NMR spectra the H_2, H_1 and H_3 protons peaks are shown (Scheme VI) at 1.40 and 1.25ppm respectively[30]. From the spectroscopic data is concluded that the polymer obtained is the **a** (Scheme VI).

The poly-(1,7-octadiene)

The possible type of polymer through the polymerisation of 1,7-octadiene[32] is shown in Scheme VII.



a

Scheme VII

The polymer **a** has to be excluded because the polymer obtained is insoluble in any organic solvent and it is a crosslinking polymer. The IR spectra of crosslinking poly- (1,7-octadiene) is simple and shows the methylene absorbance at 2920cm^{-1}, 2840cm^{-1} and 1460cm^{-1}. Traces of double bond at 1640cm^{-1} and weak absorptions of 1,4 trans, 1,2 trans and 1,4 cis at 990cm^{-1}, 910cm^{-1} and 710cm^{-1} respectively assigned to the polymer **a**, indicate that the resultant crosslinking polymer incorporates the polymer **a**.

UV spectrophotometric studies

Further proof for the cationic character of this catalyst comes from UV spectra where isomerisations of double bonds to conjugated dienes and trienes are observed due to the cationic species produced from the system $Cp_2TiCl_2/Et_3Al_2Cl_3$.

The results of the UV spectrophotometric studies of some of the polymers obtained are summarised in Table VII.

The observed conjugation in Table VII is due to the cationic species produced in the reaction mixture[44]. This is a further proof that cationic species initiating the cationic polymerisation of our monomers from the catalytic system $Cp_2TiCl_2/Et_3Al_2Cl_3$ are produced.

Table VII. UV study of polydienes

Run	Polymer	Absorbance, λ_{max} (nm)	
		conjugated diene	conjugated triene
1.	1,3-COD	216	259
2.	cis-1,3-pentadiene	217	—
3.	trans-1,3-pentadiene	217	—
4.	1,5-hexadiene	214	256

Aknowledgment

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ΚΑΤΙΟΝΙΚΟΣ ΠΟΛΥΜΕΡΙΣΜΟΣ ΔΙΕΝΙΩΝ ΜΕ ΤΟΝ ΟΜΟΓΕΝΗ ΚΑΤΑΛΥΤΗ ZIEGLER-NATTA $Cp_2TiCl_2/Et_3Al_2Cl_3$.

ΠΕΡΙΛΗΨΗ

Μιά σειρά διένίων, συζυγιακά ή μή, κυκλικά ή άκυκλα, πολυμερίζονται με τον καταλύτη Ziegler-Natta $Cp_2TiCl_2/Et_3Al_2Cl_3$. Για πρώτη φορά επιβεβαιώνεται με τον προαναφερόμενο καταλύτη ο κατιονικός πολυμερισμός των δικυκλο-[2.2.1]-επτα2,5-διενίου (NBDE), 1,3-κυκλοοκταδιενίου (COD), 1,3-κυκλοεξαδιενίου (CHD), cis και trans-1,3-πενταδιενίου (PD), 1,5-εξαδιενίου (HD) και 1,7-οκταδιενίου (OD). Τα λαμβανόμενα πολυμερή και ειδικά τα άκυκλα συζυγιακά διένια δεν εμφανίζουν στεροκανονικότητα και η απώλεια των διπλών δεσμών αποδίζεται σε ενδοκυκλικά, διακλαδούμενα, κυκλικά και δικτωτά προϊόντα. Ο κατιονικός μηχανισμός σχηματισμού των λαμβανομένων πολυμερών επιβεβαιώνεται με φασματοσκοπία ^1H-NMR, IR, UV καθώς και υγρή χρωματογραφία πηκτής (GPC) των προϊόντων.

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BIOSENSORS: A STATE OF ART IN CHEMISTRY

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SUMMARY

Technological advances and better communication between engineers, chemists, doctors and biologists have set the stage for rapid and important advances in the field of biosensors. This review examines the major categories of biosensors by consideration of detection schemes (electrochemical, optical, piezoelectric and calorimetric) and biological molecular recognition elements. A summary of some recent applications of biosensors in industrial, agro-food, environmental and clinical chemistry demonstrates the potential for widespread acceptance of these devices, and for uses in entirely new areas such as space science or for the construction of artificial parts of the human body. Although substantial investment in the biosensor market continues, numerous problems in moving from basic research to large scale factory production must still be overcome. Fundamental problems related to interfacial chemistry and the energetics and reversibility of molecular recognition elements are examined. The art of production of biosensors has been established by the current basic research efforts, and the achievement of the goal of fully characterized multifunctional miniaturized practical biosensors is now becoming visible.

Key words: Biosensors, Transduction, Molecular Recognition, Chemical Technology, Commercialization.

INTRODUCTION/TERMINOLOGY

In the past two decades the development of biosensors has emerged as one of the major avenues of research within the field of analytical chemistry. **Biosensors** are compact, self-contained devices for rapid and selective detection and measurement of chemical species in complicated mixtures. Such a device incorporates a biological sensing element in close proximity to, or integrated with a physical transducer or detector (Figure 1)[1].

The transducer responds to the product of the sensing process and outputs the response in a form that can be amplified, stored, or displayed. Hence, any new development in the field of physical sensors can become a tool in the development of biosensors. The **sensing element** is used to selectively transform the chemical signal (i.e. concentration of analyte) into a measurable physical signal which is processed by the detector. The selectivity is vital as it minimizes or eliminates steps that are used in conventional analytical procedures such as sample preparation and separation, and avoids the use of costly and sophisticated structural analysis systems (e.g. chromatographic/ mass spectrometric systems). Ideally biosensors are reversible devices and interfacial chemical reactions should be rapid and reversible so that cycling of analyte concentration should provide for calibration of response. However some sensors are not reversible and, in the worse case, are one-shot detectors that self-destruct or become insensitive after a single measurement; these sensors are classified as **Bioprobes**. **Actuators** are sensors or detectors which are designed to provide an active output as for example may be useful for process control. Finally, **Dosimeters** are irreversible devices that continue to accumulate and integrate a unidirectional input signal.

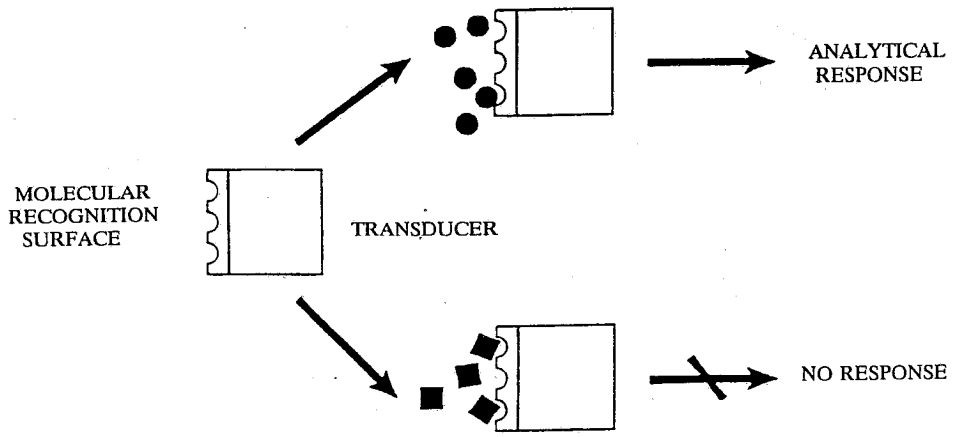

Figure 1. The biosensor: surface modified transducer which is reactive towards a specific analyte.

The first biosensor was prepared three decades ago using an enzyme in combination with an electrochemical transducer[2]. Since then the rate of appearance of papers about biosensors in the literature has increased at an exponential rate, and now there are journals that are totally dedicated to the area. The scope of this review is to examine the role of biosensor technology in the field of chemical science and its intercorrelation with other sciences. The potential uses of these devices in various fields of chemistry are included in this review, as are problems related

to perfomance characteristics that need to be solved before widespread commercialization of practical devices is achieved.

THE BIOSENSOR CONCEPT: TRANSDUCTION OF MOLECULAR RECOGNITION

Biological materials such as enzymes, immunochemicals (antibodies), portions of cells (organelles), tissues or groups of cells, and molecular receptors serve as molecular recognition elements to impart selectivity for the analyte of interest, while rejecting other compounds of similar structure or related properties. The concept of biosensing is based on the **transduction of molecular recognition** where a binding interaction or reaction between a **receptor** and a **stimulant** (analyte) defined by molecular properties of size and charge distribution results in an enhanced concentration of analyte at an interface. Transduction can be achieved by a **passive** detection scheme (i.e. no external energy is supplied to the detector) by observing changes in surface free energy associated with alterations of electrochemical potential, or by observing thermal changes associated with heats of reaction. Such passive strategies set limitations of sensitivity based on the thermodynamics of selective interactions. Improvements of sensitivity can be obtained by allowing the selective chemical interaction to modulate a large amount of energy (signal) derived from some external source in the form of electrochemical potential, electromagnetic radiation, or mechanical motion (**active** detection scheme).

Natural electrochemical chemoreceptive processes combine the advantages of passive and active sensing methods[3]. External energy is stored across a membrane as an electrochemical potential and a single binding event can generate a discharge of this energy by permitting the opening of an ion channel through the structure. Therefore there is an intrinsic amplification step of the energetics associated with the selective binding event which provides high sensitivity. The implementation of artificial chemoreception is therefore an area which is receiving significant attention.

For both passive and active biosensors, a signal develops because of changes in the structure of a receptor or its environment (e.g. complex formation, product evolution). Such signals can be used to determine concentrations, and importantly can be used to derive information about the chemical properties of a sample. Biosensors that are able to recognize taste/odourous[4] and eye irritant[5] compounds have been reported and structural information can now be derived from selective biosensors when multidimensional analysis of signals and/or multiple assemplies of linked devices are used. Theoretically, such analysis could provide information similar to that provided from techniques using expensive and complicated instrumentation such as GC/MS systems. Table 1 indicates some of the diversity of analytical situations that may benefit from biosensor technology, and illustrates how interdisciplinary the field is in terms of end users and the collaboration between various branches of science which must occur to develop useful devices.

Table 1. Applications of biosensors

General area	Typical examples
Industrial monitoring	1. Control of fermentation reactors 2. Food quality inspection (eg. fish freshness) 3. Waste product management
Continuous environmental monitoring	1. Monitoring of industrial effluents and water supplies for pesticides and chlorinated hydrocarbons 2. Narcotics and explosives, environmental detection 3. Warning of (bio)chemical release for civilian or military defence
Robotic control	1. Control of industrial environmental ventilation systems 2. Assembly line robotics requiring sense of taste/smell e.g. food quality
Clinical or invasive biochemistry	1. Continuous monitoring of drug therapy 2. Rapid screening of narcotics 3. Detection of biological markers associated with evaluation of health, or early detection of diseases(e.g. cancers) 4. Feedback control of artificial organs such as pancreas for diabetics

BASIC CATEGORIES OF BIOSENSORS

Biosensors can be classified according to their transduction device in four major categories: Electrochemical, optical, piezoelectric and calorimetric devices. Recent trends include classification schemes based on the biological recognition elements which are either molecular, or represent whole cells or portions thereof. Table 2 summarizes the common classification schemes that are used to define biosensors.

Electrochemical Biosensors

The most commonly used electrochemical biosensor systems operate in a "passive" potentiometric mode (ion selective electrode, ISE) or in an "active" electrolytic mode (i.e. amperometry). Conductometric biosensors based on measurement of chemically induced conductivity changes have been reported, but suffer from selectivity problems. A typical electrochemical biosensor would consist of a polymeric sensing membrane containing a selective reagent such as an enzyme, interfaced to a measurement device such as an ISE or platinum electrode. The enzyme is either covalently bound onto the electrode surface (which results in improved enzyme stability) or physically trapped near the electrode surface.

Table 2. Biosensor classification schemes		
Based on the principal biosensor output mode	Electrochemical	Potentiometric, amperometric, conductimetric, capacitance, field effect transistors
	Optical	Absorbance, fluorescence/ /chemiluminesce, fiber optics
	Piezoelectric	Bulk and surface acoustic waves
	Thermal	Thermistors
Based on the biosensor recognition element	Molecular	Enzymes, antibodies, receptors
	Cellular	Organelles, whole cells, tissue slices

Most reports of potentiometric enzyme electrodes have been confined to substrates for deaminases (NH_3/NH_4^+ system), decarboxylases (CO_2 detection) and hydrolases. Biosensors based on oxidase enzymes have received the greatest attention in amperometric investigations:

$$\text{Substrate} + O_2 \xrightarrow{\text{Oxidase}} \text{product} + H_2O_2 \qquad (1)$$

allowing monitoring of O_2 consumption at an (Clark) oxygen electrode, or of H_2O_2 production at a positively polarized electrode:

$$H_2O_2 \xrightarrow{+650\ \text{mV (vs. Ag/AgCl)}} O_2 + 2H^+ + 2e^- \qquad (2)$$

Usually the amount of enzyme used is in a large excess with respect to substrate concentration so that response becomes limited by mass transport phenomena and independent of the amount of enzyme. The signal derived at steady-state conditions, or the rate of change of a signal, can be used to determine the concentration of the monitored species. Coupled reactions of two or more enzymes and/or substrates can extend the variety of analytes that can be measured, and can improve the sensitivity of analyses from μM to nM detection levels.

Antibody-antigen interactions can be monitored if either of the binding species is tagged with an enzyme in strategies which involve sandwich assays or competitive binding assays (enzyme-linked immunosensors)[6]. The drawbacks in the use of labelled species and immunological reactions are that an extra step(s) makes the method more tedious and time-consuming, and the selective chemistry is generally not reversible. Immunopotentiometric systems able to directly monitor an immunological reaction at an electrode surface have been proposed[6], but are not practical in complex matrices where mixed potentials cause substantial interference.

Present limitations of electrochemical devices include the necessity for stable reference electrodes, electrical noise

susceptibility, non-selective adsorption and surface occlusion leading to drift of surface activity, evolution of mixed potentials, Debye length effects (which lower the effective charge of interactions associated with larger biological receptors extending into the sample solution), and ionic strength, temperature, and pH sensitivity.

Semiconductor devices for ion concentration determinations which were introduced in 1970 by Bergveld[7], have extended the applications of potentiometric biosensors. A representative device is the so called **chemically selective field-effect transistor** (CHEMFET) or **ion selective field-effect transistor** (ISFET) (if the monitored species is an ion), where the gate of a field effect transistor is replaced with a conventional potentiometric membrane supported on an insulator (Figure 2).

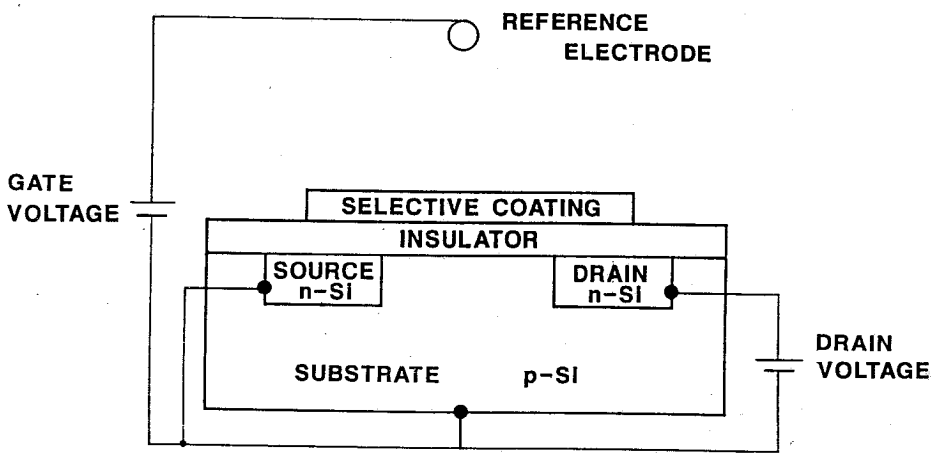

Figure 2. A schematic representation of a CHEMFET showing chemical selectivity at the "gate region".

Modulation of the gate voltage by a biochemical reaction can control an electronic current within the transistor between the "source" and "drain" in a configuration which typically has a size of only a few square millimeters. This arrangement permits direct conversion and amplification of the potentiometric signal into an electronic current of low noise. Semiconductor technology has made possible the development of a sensor which is capable of microelectronic signal processing and control in a single miniature package suitable for work in small volumes, and even for short-term *in vivo* implants. The majority of enzyme-based FET devices (ENFETs) have used a transduction scheme based on potentiometric monitoring of hydronium ion activity. This sets a fundamental limit to the type of enzyme that can provide an analytical signal. Efforts to overcome this problem have resulted in the development of devices such the ammonia-sensitive iridium-metal oxide semiconductor system[8].

Semiconductor devices for biosensor applications usually use

potentiometric methods, and are therefore limited by the problems previously listed. Further obstacles originate from device instability in liquid media, and electrical noise sensitivity. ISFETs that operate by use of a differential mode have been reported recently[9] where a dual pH FET gate is prepared with active enzyme and inert (albumin) cross-linked layers. Comparison of the signals at the two gates can compensate for pH and temperature effects, but not for variations in sample buffer capacity (which sets the limits of these devices for *in vivo* applications).

The **light addressable potentiometric sensor** (LAPS) exploits the H^+ sensitivity of silicon nitride without encapsulation to protect the device in solution. The basis of operation of LAPS biosensors is that an appropriate bias potential is applied to a silicon plate, which has a nitride layer surface exposed to solution (Figure 3)[10]. An alternating photocurrent is created within the silicon by means of a (modulated) light emitting diode, and the presence of this current effectively completes a measurement circuit which runs through the silicon, silicon nitride and external solution compartment. Brief periods of activation of the circuit permits observation of the total bias potential, a variable component of which is solution pH as modulated by selective enzyme-substrate reactions. The device is possibly the most successful commercial electrochemical biosensing system presently available, and is commonly used to detect antigens by an enzyme-amplified immunochemical sandwich assay, and more recently has been adapted for deteminations of whole cells.

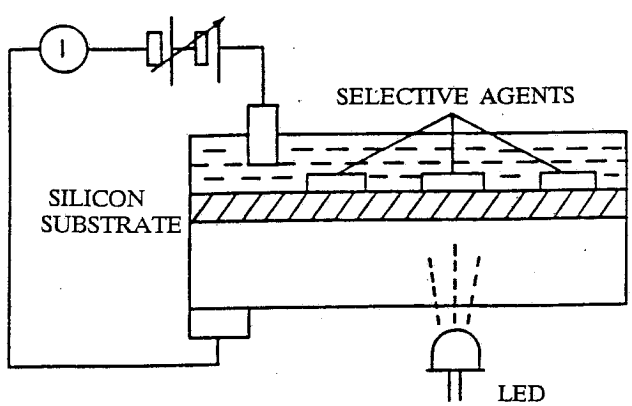

***Figure 3.** A schematic representation of a LAPS device.*

Optical Biosensors

Optical properties which can be used for sensor construction include absorption, fluorescence, chemiluminescence, reflectance, scattering and refractive index. Significant innovation has been achieved by the use of fiber-optic technology, where **optical fibers** transmit information to/from a remote compartmentalized

target reaction. The sensing layer is either physically trapped or chemically bound at the terminus of the optical fiber (extrinsic sensor), or is immobilized as a cladding which surrounds the optically conductive core thereby permitting optical transduction by means of an evanescent wave (intrinsic sensor, Figure 4). Sensors which use optical fibers are referred to as **optrodes** (optical electrode) or **optodes** (from the Greek: the optical way).

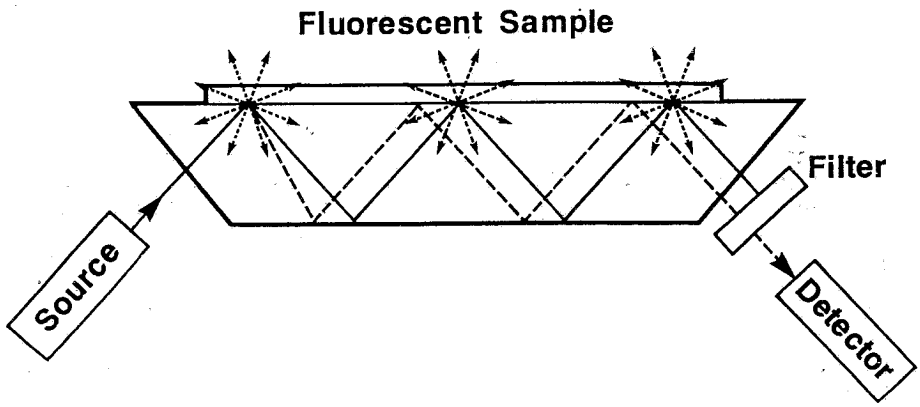

Figure 4. *An intrinsic fiber-optic sensor showing a chemically selective coating on the waveguide surface being excited by an evanescent wave.*

The sensitivity of optical biosensors is greatly increased when fluorescence or chemiluminescence detection schemes are used. Fluorescence processes are unique as they offer the possibility of multidimentional analysis by concurrent observation of wavelength, intensity, polarization and event lifetime. In combination, these analytical parameters can be used to define unique solutions to both qualitative and quantitative aspects of analysis.

The multidimentional approach allows optical systems to be self-correcting with respect to activity drift and non-selective interference (and therefore could be self-calibrating), and can potentially obviate many of the problems associated with other device categories. However fundamental problems such as loss of binding activity and reversibility of selective proteins remain, and signal-to-noise limitations of instrumentation (particularly with respect to scatter and electronic noise) can be problematic at lower concentrations of emitter.

Piezoelectric Biosensors

Piezoelectric devices are mainly based on AT-cut quartz crystals that can function as a quartz "microbalance". Crystal

oscillation based on motion between parallel crystal planes (thickness shear) at a resonant frequency is initiated and maintained by means of an external AC voltage. Sauerbrey[11] showed that the frequency change (Δf) is related to any added mass (Δm) as follows:

$$\Delta f = -2f_0^2 \, \Delta m / A \, (\mu_q \, \rho_q)^{1/2} \qquad (3)$$

where f_0 is the resonant frequency of the crystal in the absence of a mass loading on the surface, μ_q is the shear modulus, ρ_q the density and A the surface area of the (active) crystal face. A number of basic assumptions underlie equation (3) and various modifying theories have been proposed for deviations from ideal operation (which are particularly relevant to operation in liquids)[12].

Mass detection with piezoelectric sensors can be achieved by using two modes of operation:

1. **Surface acoustic wave** (SAW) devices have two sets of interdigitated electrodes on one interface of the piezoelectric material (ST-cut quartz). One set of electrodes generates local deformations in the quartz, which are transmitted as mechanical waves to a receiver electrode array (Figure 5a). An interaction of the launched wave with any extraneous material deposited at the surface of the quartz changes SAW speed and amplitude, thus enabling quantification of the deposited mass. SAW devices are used as gas sensors, and have been investigated in the liquid phase where problems arise due to damping phenomena.
2. **Bulk acoustic wave** (BAW) devices are operated by application of the oscillating electrical field between metal electrodes located at both crystal faces, i.e. along the thickness dimension (Figure 5b). Given appropriate crystal symmetry and orientation, either a shear (most common design of the resonator) or a longitudinal wave, propagating along the thickness dimension, will be produced.

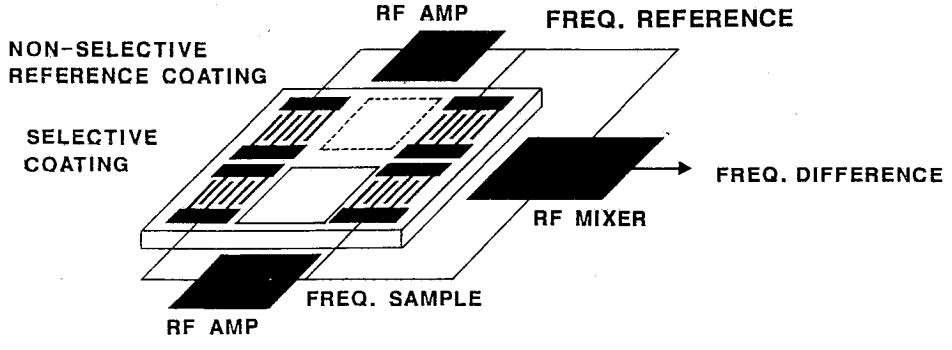

Figure 5a. A schematic representation of a SAW device.

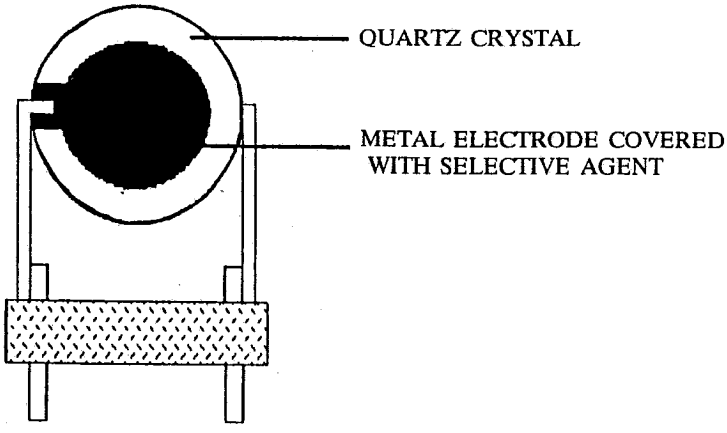

Figure 5b. *A bulk wave resonator.*

The mechanical oscillation of a piezoelectric crystal can be perturbed by mass and microviscosity alterations caused by selective binding reactions at the surface of the crystal. Frequency changes smaller than 1 Hz can be measured reproducibly, providing ng sensitivity or better for BAWs with respect to adsorption of mass to the surface of the device. Evolution of products from a reaction is not necessary for transduction to take place, as shown by studies of antibodies on one surface of the device[13]. Remote sensing may be possible with these devices, since the frequency response information is not necessarily distorted by extraneous electrical or magnetic noise. A multidimensional approach is possible using a network analyzer which can resolve various modes of oscillation within a crystal. Mass and viscosity effects may be resolved by study of the different oscillation modes, and therefore it may be possible to quantitatively identify non-selective response and noise. The piezoelectric devices offer significant practical advantages in contrast to electrochemical and optical biosensors by virtue of their extremely sensitive and accurate response to mass and viscosity alterations. However response to such general physical changes implies serious potential difficulties due to non-selective interferences.

Calorimetric Biosensors

Most enzyme reactions are sufficiently exothermic to allow for calorimetric detection. Enzyme reactors which incorporate thermistors have been developed for monitoring a wide range of substrates such as urea, glucose, ethanol, and penicillin[14]. However, the temperature changes which are generated are in the milli-centigrade range even with quantitative conversion of relatively large quantities of substrate to product. The thermal enzyme probe (TEP) uses enzyme in close proximity to the thermistor sensor surface, but still has a low temperature yield. Recent research has been directed towards amplification schemes

by substrate recycling, and re-design and optimization of the apparatus to allow determinations of substrate in the nM range.

Special Bioreagent Systems

Most biologically active species such as enzymes and molecular receptors rapidly lose biochemical activity when they are removed from their natural host matrice. This disadvantage has directed research towards the use of whole tissues or cell suspensions. Microbial and eukaryotic cell- and tissue-based biosensors have been studied, as well as intact chemoreceptor organs present in some animal species. The so-called banana[15] and crab electrodes[16] have been used to monitor urine constituents and amino acids, respectively, and a variety of similar biosensors have been reported[17]. Drawbacks of these biosensors are related to aspects of selectivity in complicated matrices, the need for maintenance of nutritional and environmental requirements, and prolonged dynamic response characteristics. This category of biosensor may be useful in situations where samples are well defined.

Natural chemoreceptive membranes consist of a bimolecular layer of lipids, which incorporate protein molecules that have molecular recognition properties, i.e. receptors. These receptor molecules are difficult to isolate and rapidly denature when removed from their natural lipid environment. In addition, some receptors operate by triggering of secondary messenger cascades and require additional transduction elements to generate a signal. However, the ion channel system that is found in biological organisms is associated with rapid, reversible, sensitive and selective chemical signalling, with unique features such as the amplification and transduction of the chemical information into an electrical pulse by switching of channel conductivity.

Artificial lipid membranes offer an opportunity for biosensor development[18, 19]. The essential idea is that a protein which can selectively bind to a specific organic or biochemical species can be incorporated into an ordered lipid membrane assembly so that selective binding events can lead to changes in the structure or electrostatic fields of the membrane (transduction). The perturbation of the membrane can be monitored by electrochemical or fluorescence detection techniques, and the membrane provides a generic transduction system suitable for monitoring enzyme, antibody and molecular receptor interactions with the underlying advantage of intrinsic signal amplification. Commercialization of these highly promising devices has not yet been achieved owing to the inherent fragility of the artifical lipid membranes, but recent work in areas of polymerization and self-assembly has indicated that this problem may soon be solved.

BIOSENSOR PERFORMANCE CHARACTERISTICS

Table 3 provides a generalized compendium of practical operational characteristics for a wide variety of biosensors. Table 3 does not include a major feature relevant to commercialization which is practical lifetime, and does not consider special needs such as sterilization. The lifetime is the

usable period of biosensor performance, either as a functional device or as a stored device with a "shelf life". The lifetime largely depends on the nature of the biosensing element and the method of immobilization, and should ideally extend to a period of months. Generally chemical immobilization of active species extends lifetime in contrast to physical entrapment. It has been shown that immobilization onto lipid membranes may stabilize certain active proteins[20].

Table 3. Functional Characteristics of Biosensors

Feature	Requirement	Examples/Comments
Selectivity	Ideally specific	Highly purified enzymes and monoclonal antibodies.
Sensitivity	A linear calibration curve is preferable	ISE exhibit a semilogarithmic response.
Detection limit (D. L.)	It should be better than nM range	Potentiometric systems have μM D. L. Fluorescence offers the lowest D. L.
Reversibility	Recovery of full analytical response is essential	General Reversibility: enzymes> molecular receptors> antibodies
Response time	99% maximum signal development within a few seconds	Related to analyte concentrations and diffusion phenomena.
Size	miniaturized systems are preferable	Carbon fibers offer the route for microfabrication.
Ruggedness	Insensitive to minor physical or electrical shock; no calibration drift	Solid state systems and covalently bound chemistry preferred.
Reliability	Self-calibrated system; easily used by untrained personnel	Fiber-optic systems can be self-calibrated.
Cost	Low cost for disposable or continuous widespread use	Possible advantage for lithographic technology using semiconductor or piezoelectric devices.
Signal recovery	Signal must be free from electromagnetic interferences and easily transmitted	Fiber-optic devices can be useful for remote distributed biosensor networks.

An overview of some of the most pressing areas of fundamental research that are required to improve biosensor

performance characteristics include:
1. understanding and manipulation of the energetics of molecular binding, with particular emphasis on reversibility and selectivity
2. direct transduction of binding events
3. direct physicochemical methods of signal amplification
4. quantitative measurements of interferences, and
5. stimulated reversibility for generation of active sites

USES

Some examples of problems that can be solved by biosensors are now given to indicate how these devices may find practical application.

Many biotechnological processes involve fermentation reactions that produce ethanol. In beer or wine industries continuous monitoring of ethanol is desirable. A significant number of biosensors have been proposed, and are generally based on enzyme reactions such as the alcohol-oxidase catalyzed oxidation of ethanol, using amperometric detection for example.

$$C_2H_5OH + O_2 \xrightarrow{\text{Alcohol oxidase}} CH_3CHO + H_2O_2 \qquad (4)$$

A combination of potentiometry and a selective biochemical reaction can be used to solve the routine industrial problem of analysis of the low-calorie sweetener aspartame (N-L-a-aspartyl-L-phenylalanine 1-methyl ester) in vanilla pudding, root beer, Crush, Coca-Cola, and diet sweetener powder/tablets[21]. Aspartame is produced from L-aspartic acid and phenylalanine and is usually determined in these food products by slow, labor-intensive chromatographic methods. The bioenzymatic potentiometric method for the determination of aspartame uses the following coupled enzyme reactions:

$$\text{Aspartame} \xrightarrow{\text{carboxypeptidase A}} \text{phenylalanine} + \text{L-aspartic acid} \qquad (5)$$

$$\text{L-aspartic acid} \xrightarrow{\text{L-aspartase}} \text{fumarate} + NH_4^+ \qquad (6)$$

Recent reports have investigated piezoelectric devices in combination with immobilized antibodies to monitor volative environmental contaminants such as certain pesticides and narcotics[22]. Valproic acid antiserum and antibody against parathion were included among a set of proteins used, and responses to the pesticide were confirmed.

Other application areas where biosensors are set to make a significant impact include the established needs of medicine and veterinary science, where access to biochemical information has always been at a premium. Biosensors for *in vivo* applications have been reported. A recent design for an implantable oxidase based glucose biosensor has deployed the electrode along the needle shaft rather than at the tip to facilitate membrane coating[23]. Urea, penicillin and acetylcholine are among the most important chemicals of clinical interest. Potentiometric enzyme electrodes with response times of a few seconds have recently

been reported[24]. These were prepared by the deposition of ultrathin (1-2 μm) enzyme layers over glass pH electrodes by cross-linking surface-deposited enzyme with a spray coat of glutaraldehyde.

The development of biosensors for the direct monitoring of antibody-antigen interactions has been the subject of many studies. Direct potentiometric analysis of immunological interactions is of limited practical utility owing to interference from mixed potentials. Immunochemical interactions can activate a complicated protein system, known as the complement system, to lyse sealed microcapsules (vesicles) containing an electroactive marker[25].

Future utilization of biosensors may include applications in biomedical experimentation (e.g. monitoring the health of crew members on space missions) and monitoring of water recycling during long-term space missions[26]. Experiments could include monitoring metabolites (e.g. glucose, lactic acid, creatinine, cholesterol), enzymes (e.g. alkaline phosphatase), hormones (e.g. adrenaline, cortisol, growth hormones). Biosensor units could also be useful for monitoring the purity of recycled water during long-term space missions or after processing for environmental purposes. Various parameters such as microbial count, presence of toxics like ammonia, and hydrocarbons, could be monitored.

Some researchers are now attempting construction of an artificial neural network by use of living cells[27]. Even if these efforts are still far from construction of an artificial brain, the implication of a combination of neural networks with distributed sensor arrays is that artificial chemoreceptive systems may become available.

THE CONSUMER ROLE

New classes of biosensors are emerging from research labs to become new products in the marketplace. Some will pose competitive threats to established products, while others will satisfy new market requirements where no suitable biosensors are yet available. Biosensor research is fuelled primarily by potential markets in agriculture, the environment and medicine. It is estimated that there will be a global biosensor market of $ 350M by 1996, and possibly of $ 750M by the year 2000[28].

Typical examples of applications of commercialized sensing approaches in agro-food industries and in invasive clinical monitoring were reported recently[29]. These included testing kits to rapidly identify adulteration of beef with kangaroo or horse meats based on the use of antibodies. Antibodies (or DNA probes) were also the basis of a new immunodiagnostic biosensor that identifies different substances in a drop of blood, and will soon be able to test for new varians of HIV[29].

Problems such as bioincompatability have stimulated the biosensors community to investigate a wide variety of surfaces and materials. Awareness of materials science has had a surprising impact on biosensor design, and is providing an impetous for movement from conventional "dip" style sensors to much more sophisticated products.

Some problems that plague development of worldwide

commercialization are based on the need for industry-wide standards for biosensor testing and statements of performance characteristics. Communication barriers between chemists, engineers, physiologists and biologists tend to dissipate by means of workshops, symposia, personal communication and collaborations, and many more interdisciplinary meetings are required. Surveys and studies are starting to appear indicating that terminology describing biosensor performance characteristics should be established.

FUTURE TRENDS

While biosensors have been proposed as systems with great potential to revolutionize analytical measurement technology in areas such as clinical, environmental and agro-food analysis, it is clear that there has been only limited success in commercialization. While biosensor technology does not lack on the device side, deficiencies are prevalent in the areas of overall compatible marriage of selective biochemistry with device structures, and in the development of environmentally rugged receptors.

Of frustration to biosensor specialists is the fact that protein engineering technology can provide a host of selective binding agents, yet these species were not designed by evolutionary processes to operate in chemoreceptive communication processes. This has resulted in an imaginative array of analytical methods which are designed to create and amplify an analytical signal, viz. sandwich and competitive assays and enzyme-labelling. The energetics of the immunochemical binding interaction, and the need for secondary labelling by the introduction of secondary reagents has severey limited the use of immunochemical reagents as effective chemical matrices for development of continuous biosensing devices. Of further frustration is that the practical manipulation and immobilization of proteins often leads to significant denaturation which tends to progress with time. The resulting long-term drift and degradation of chemical activity, coupled with non-selective interactions associated with the presence of a protein at an interface have made it very difficult to fabricate a reliable monitoring device. It is clear that as long these problems are not solved, it will not be possible for biosensor technology to fully provide opportunities that could revolutionize analytical methodology. However there is considerable optimism that these issues will be addressed and overcome. A number of important advances are distributed throughout the chemical literature, but have not been critically compiled and analyzed, including: direct detection of selective binding interactions of antibodies using artificial chemoreception with intrinsic amplification, the genetic engineering of molecular structure to place fluorescent tags at only the binding site, the molecular wiring of an active site to an external circuit, immobilization of antibodies onto Teflon to reduce denaturation, and applications of chemometric concepts such as neural networks to provide calibration and to account for interferences. It is imperative that such new ideas and technology be pursued by the analytical chemistry community if biosensors are to reach their full technological potential.

ΠΕΡΙΛΗΨΗ

ΒΙΟΑΙΣΘΗΤΗΡΕΣ: Η ΤΕΛΕΥΤΑΙΑ ΤΕΧΝΙΚΗ ΕΞΕΛΙΞΗ ΣΤΗ ΧΗΜΕΙΑ

Οι πρόσφατες τεχνολογικές εξελίξεις και η καλύτερη επικοινωνία μεταξύ χημικών, φυσικών, ηλεκτρονικών, ιατρών και βιολόγων έθεσαν τις βάσεις για ραγδαίες και σπουδαίες αναπαλύψεις στο πεδίο της επιστήμης των βιοαισθητήρων. Το παρόν άρθρο ανασκοπήσεως εξετάζει τις διάφορες κατηγορίες των βιοαισθητήρων με βάσει τα σχήματα ανιχνεύσεως (ηλεκτροχημικά, οπτικά, πιεζοηλεκτρικά και θερμιδομετρικά), καθώς και με τα μοριακά βιολογικά στοιχεία αναγνωρίσεως. Μία περιληπτική ανασκόπηση των πρόσφατων εφαρμογών των βιοαισθητήρων στη βιομηχανική, περιβαλλοντολογική και κλινική χημεία, στη χημεία τροφίμων και γεωργική επιστήμη αποδεικνύει τις δυνατότητες των συσκευών αυτών, των οποίων οι χρήσεις επεκτείνονται ακόμη και στην επιστήμη της αστροναυτικής ή για την κατασκευή συνθετικών μερών του ανθρώπινου σώματος. Οι σημαντικές επενδύσεις στη βιομηχανική κατασκευή των βιοαισθητήρων συνεχίζονται με μεγάλο ρυθμό· εν τούτοις ο αριθμός των προβλημάτων που πρέπει να λυθεί, ώστε να γίνει μετακίνηση από τη βασική έρευνα στη μαζική παραγωγή σε βιομηχανικά κλίμακα, παραμένει ακόμη μεγάλος. Τα αντικείμενα της σημερινής έρευνας σχετίζονται με τη χημεία μεσεπιφανειών και την θερμοδυναμική και αντιστρεπτότητα δράσεως των μοριακών βιολογικών στοιχείων αναγνωρίσεως. Οι βάσεις της τέχνης της δημιουργίας των βιοαισθητήρων έχουν όμως ήδη τεθεί με τις πρόσφατες προσπάθειες βασικής έρευνας και η πραγματοποίηση του απώτερου σκοπού που είναι η παραγωγή ενός πλήρως χαρακτηρισμένου πολυλειτουργικού μικροσκοπίκου πρακτικού βιοαισθητήρα γίνεται τώρα ορατός.

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SEMISYNTHETIC APPROACHES FOR MORE EFFECTIVE ANTIBIOTICS

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Seminar at the University of Thessaloniki , Thessaloniki, Macedonia - Greece, dedicatted to Prof. K.L.Rinehart , University of Illinois- Urbana Ill., for his research contribution in the field of aminocyclitol antibiotics

SUMMARY:

Our approach for making better semisynthetic antibiotics are the following:
A. Preparation of semisynthetic antibiotics invulnerable by the modifying enzymes of the resistant strains of bacteria and thus active against them.
B. Preparation of hybrid, or mixed structure, antibiotics with wider spectrum of antibiotic activities, that is, aminoglycoside x β-lactams and aminoglycoside x quinolones

KEY WORDS: 5'-Epi-Neamine, β-lactam and quinolone conjugates with aminoglycosides, Aminoacid conjugates with Kanamycin and Netilmicin

The use and overuse of antibiotics resulted in the development of resistant strains bacteria. The two main categories of antibiotics in clinical use to day are β-lactams and aminoglycosides. Since these are not active against the resistant strains of bacteria, there is an urgent need of discovering new compounds of better antibiotics active against the resistant strains of bacteria.

It is believed that antibiotics such as aminoglycosides develop their antibiotic activity through their binding to the ribosomes thus inhibiting the protein synthesis of bacteria. Modifying enzymes[1] of resistant strain of bacteria are considered to be responsible for preventing the aminoglycoside either from entering to the cell or from binding to the ribosome. Considering that both procedures consist essentially of aminoglycoside binding to several proteins (transport or ribosomal) we can say that generally speaking, these enzymatic modifications aim at preventing the aminoglycoside from binding to proteins.

Our approach for making better antibiotics has been the following:

A) Preparation of modified or semi-synthetic antibiotics invulnerable by the modifying enzymes of the resistant strain of bacteria.

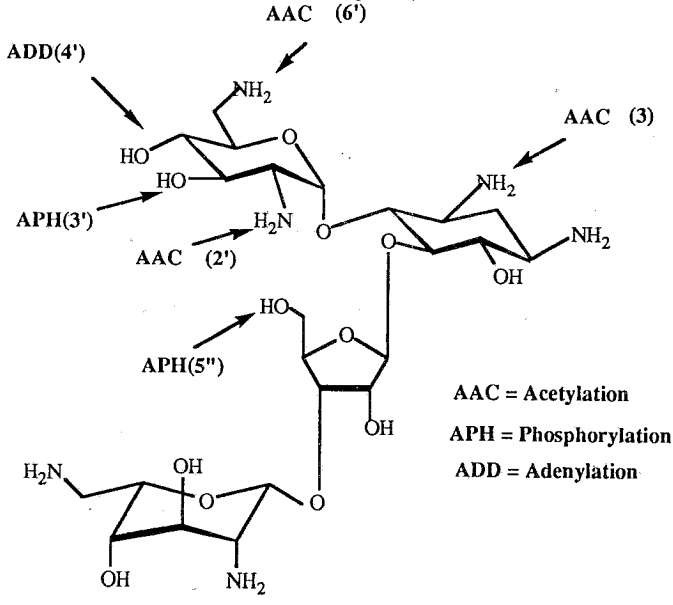
B) Preparation of "hybrid" or mixed structure antibiotics[2], with wider spectrum of activity, that is, β-lactams x aminoglycosides and quinolones x aminoglycosides.

Designing the above class of semisynthetic antibiotics A and B we have aimed not only at more active antibacterials but also at less toxic ones.[3]

APPROACH A

I. Whereas, resistance to antibiotics such as β-lactams is due to the presence of one type of enzymatic modification (β-lactams), resistance to the aminocyclitols has been shown to involve any of several different enzymatic modifications that include O-phosphorylation, O-adenylation or N-acetylation. To date, some 12 different enzymatic modifications have been characterized in clinical isolates of Gram negative and Gram positive bacteria.[1]

The structural modifications occur at several of the hydroxy- and amino- groups as exemplified in the case of Neomycin B (Figure 1).



Enzymatic modification of neomycin B by resistant strains

Figure 1

Inversion of chirality on the carbon whose substituent is attacked by modifying enzymes, may produce compounds invulnerable by the modifying enzymes. More specifically inversion of chirality at the (C-5') carbon of the neomycin B (ring A, scheme I) may produce indeed an invulnerable molecule by the modifying enzymes. This modification may be an appropriate one since neomycin B is about ten times more active than neomycin C which bears the (C-5') epimeric D-glucopyranosyl structure (Figure 2).

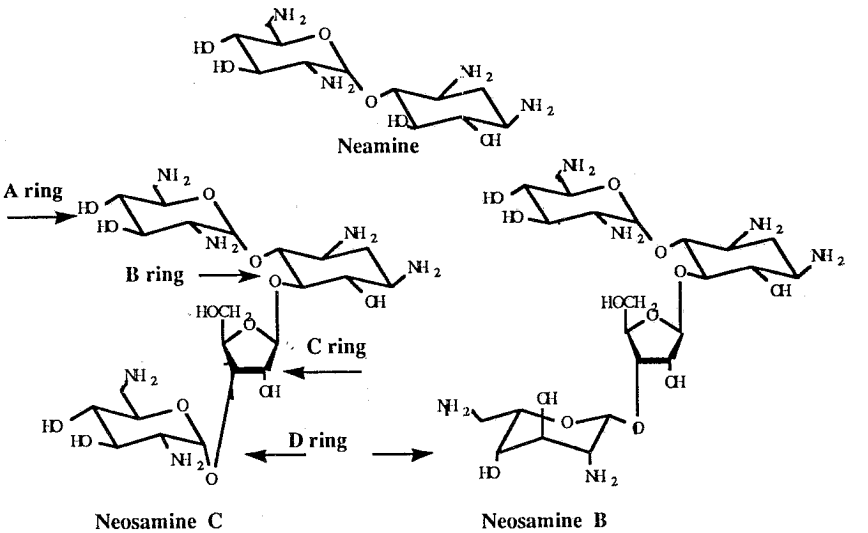
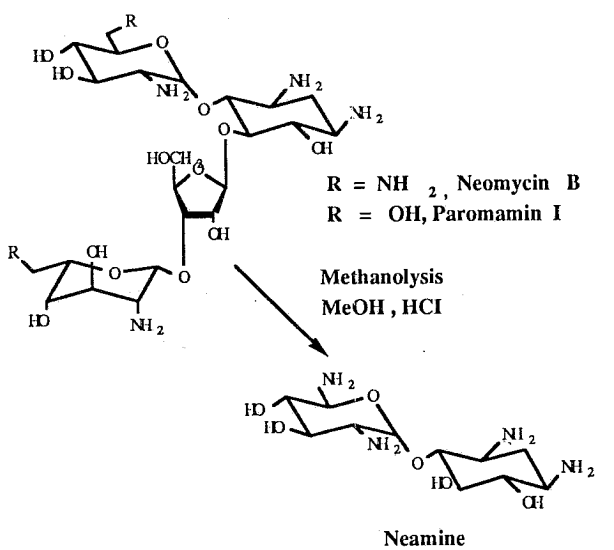


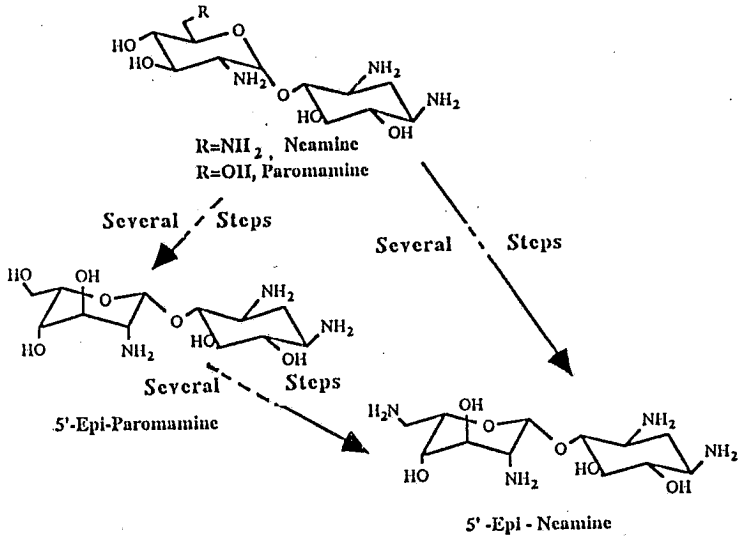
Figure 2

Although all four ring of neomycin are essential for its activity, it is a fact that the L-do-configuration of the D ring enhances the antibacterial activity of neomycin B.



Scheme I

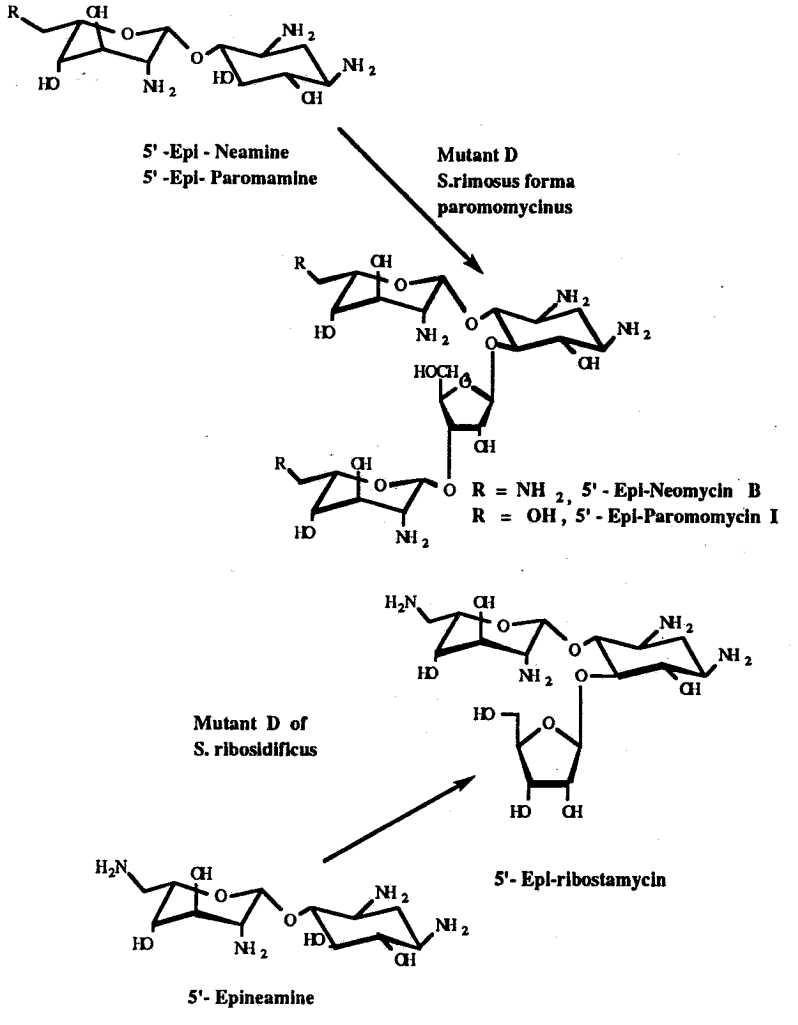
Inversion of chirality on one carbon out of the twenty three carbons of the neomycin B is not an easy task, that is why, we have focused our attention towards neamine (see Sceme I) which is a biosynthetic building block of neomycin B as well as of other aminocyclitol antibiotics. Studies of the biosynthesis of aminocyclitol antibiotics, have led to the development of the mutasynthetic technique for the preparation of related antibiotics (Neamine is part or common moiety practically of most aminocyclitol antibiotics)[4].



Scheme II : Targeted mutasynthons

The term mutasynthesis refers to a technique for the synthesis of new antibiotics by the use of specially selected mutants of strains of microorganisms which normally produce antibiotics. The mutants used are selected for their inability to produce their usual antibiotic unless one of the component parts (building block) of the usual antibiotic is added to the fermentation medium. Such mutants, which are clearly deficient in their ability to synthesize that component, have been reffered to as idiotrophs. In keeping with the term mutasynthesis[4] for the procedure, the related compounds supplementing the medium are referred to as mutasynthons and the new antibiotic as a mutasynthetic one.

Conversion of neamine to C-5'epi-neamine may produce an excellent synthon for preparing C-5'epi-neomycin B, C-5'epi paromamine, C-5'epi ribostamycin as well as there aminocyclitol antibiotics by mutational biosynthesis or even by chemical synthesis.

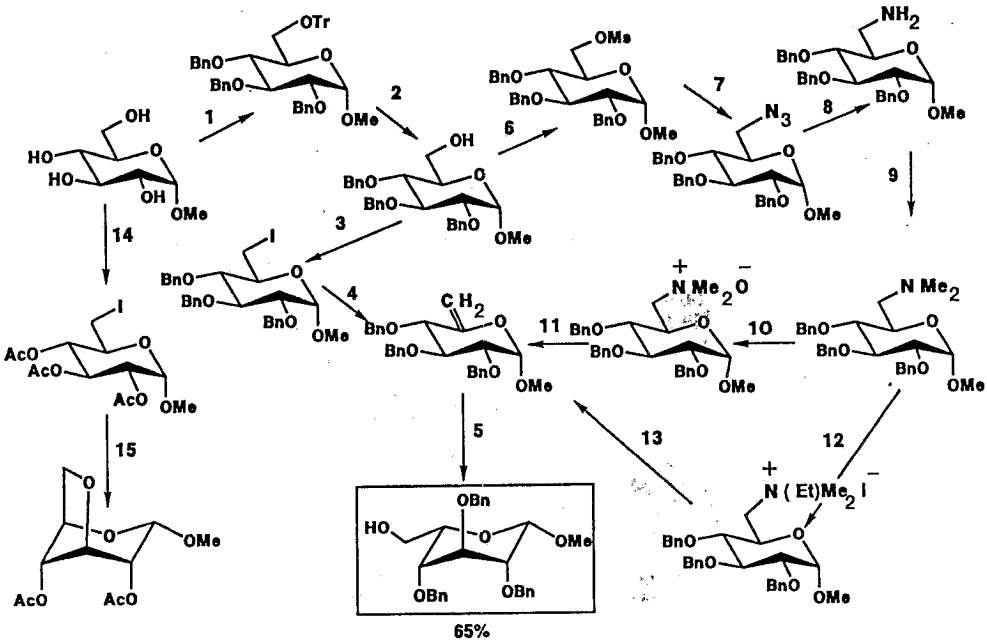


Scheme III

Prior to the synthesis of our targeted mutasynthons we have used simple molecule as models for gaining chemical experience. Model works with monosaccharide analoques for the preparation of C-5'epi-neamine or C-5'epi-paromanime are depicted below in schemes IV.

Hydroboration of methyl-α-D-xylo-exen-5-pyranoside gave 16% (scheme IV) of the undesired methyl-α-D-glycopyranoside since the hydride attack was possible from either α and β site. On the other hand when methyl-L-idopyranoside was converted to its 6-amino-6-deoxy-derivative an unusual epimerization at C-1 occurred was attributed to the conformation adopted by the L-idopyranosyl structure in solution. The above two undesired complications were not observed in analogous procedures that is hydroboration of the C' 5'-6'- unsaturated key intermediate received either from paromamine or neamine because its stereochemistry and rigidity favors the formation of C-5'epi-paromamine. However the latter compound needs further work to be converted to C-5' epi-neamine the targeted synthon (see scheme V)[5b].

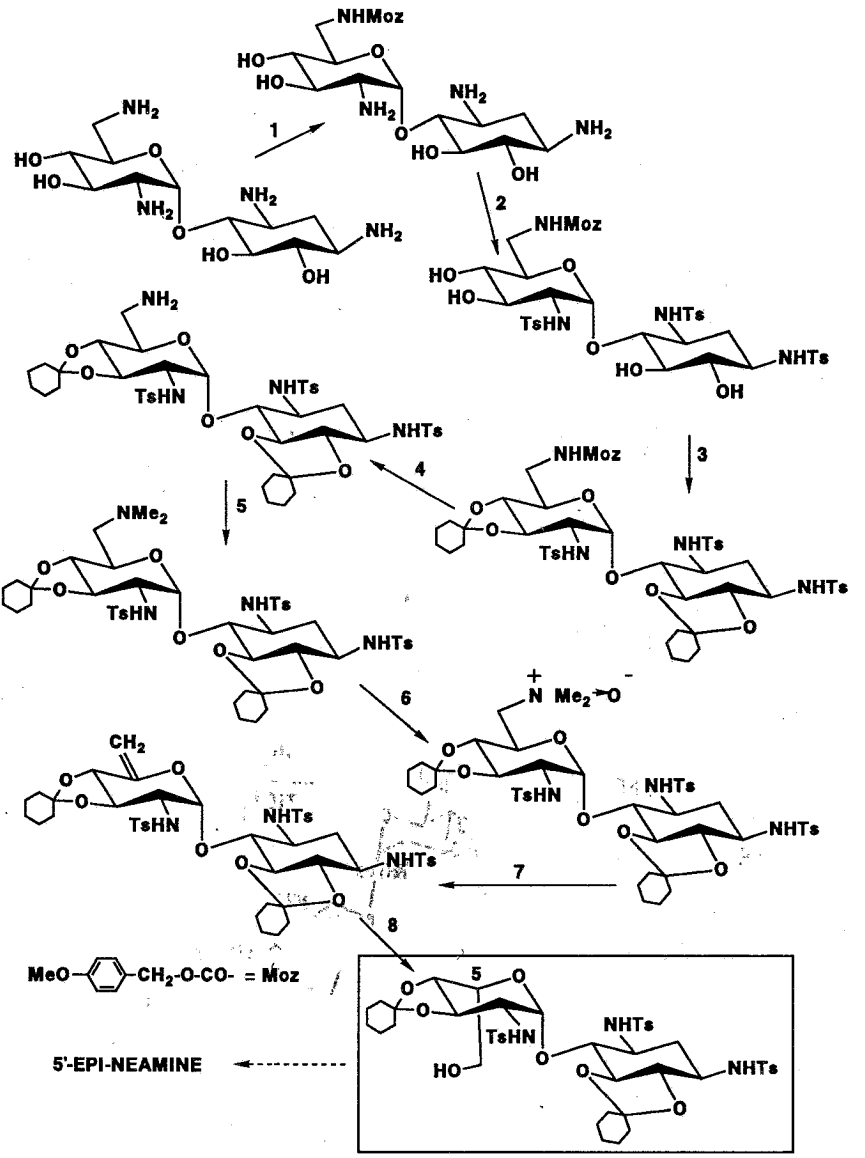
MODEL WORKS FOR C-5' EPIMERIZATION OF PAROMAMINE AND NEAMINE[5a]



1. a) TrCl/Py b) BnBr/NaOH (powder)/Bu_4NHSO_4 / C_6H_6, reflux 2. HCOOH / ether 3. $P\Phi_3$ / I_2 / Imidazole, Toluene, 70° C 4. NaO (powder) / Bu_4NHSO_4 / C_6H_6, reflux 5. a) B_2H_6 b) H_2O_2 / OH 6. MsCl / Py 7. NaN_3 / DMF 8. H_2/Pd , AcOEt 9. $(HCHO)_n$ / MeOH $NaCNBH_3$ 10. Davis' reagent 11. pyrolysis (150) 12. EtI / EtOH reflux 13. Ag_2O / MeOH / H_2O , 60°C 14. a) $P\varphi_3$ / I_2 / Imidazole, Toluene , 70° b) Ac_2O / Py 15. Bnbr/ NaOH (powder) / $BuNH_4SO_4$, C_6H_6 , reflux.

Scheme IV [5a]

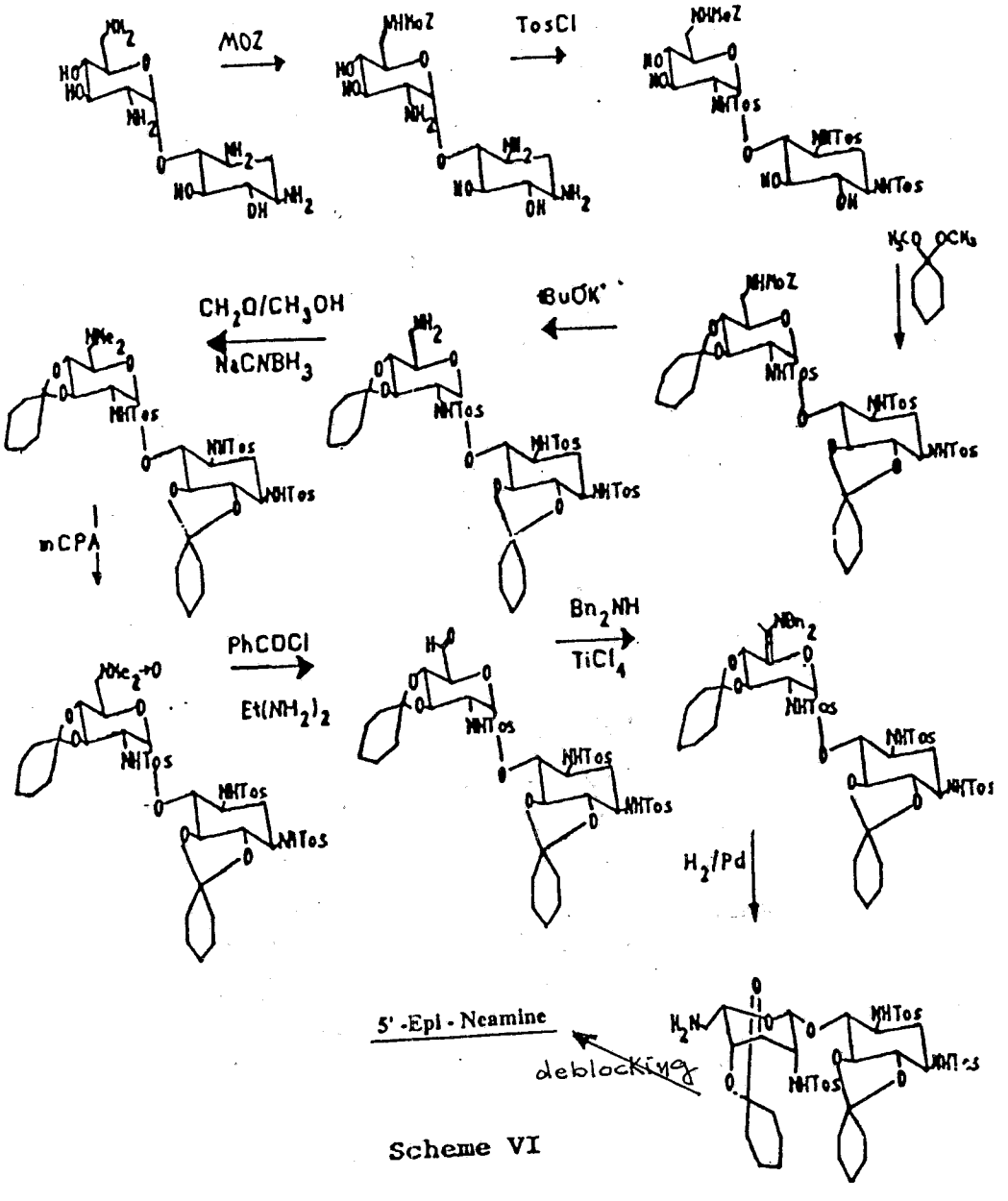
THE (C-5')-EPIMERIZATION OF PAROMAMINE[5b]



1. Moz / dioxane / H_2O 2. TsCl / dioxane / H_2O 3. 1,1-dimethoxy-cyclohexane / TsOH / DMF, 70°C 4. t-BuO K / H_2O / MeOH 5. $(HCHO)_n$ / MeOH / $NaCNBH_3$ / 6. Davis' reagent 7. pyrolysis , 150°C 8. a) B_2H_6 b) H_2O_2 / OH .

Sheme V

Thus conversion of the 6'-aminogroup (6'-NH_2) of neamine, a readily available inexpensive starting material, to an aldehydic group, subsequent enamine formation followed by hydrogenation-hydrolysis yielded the desired C-5' epi-neamine in fewer steps and much higher yield (see scheme VI)[5c].



Scheme VI

A. II Chemical derivatization of the functionalities of aminoglycosidic antibiotics which are attacked by the modifying enzymes will produce antibiotics invulnerable by these enzymes on the same functional group of these molecules. If such a derivatization could produce at the same time a molecule with better or equal antibacterial activity than the original one, then a better semisynthetic antibiotic will be produced.

Searching on the above approach for a simple way to prepare antibiotics against the resistant strains of bacteria and on the other hand having in mind that the clinical use of amino-glycosidic antibiotics is limited because of their oto-and nephrotoxicity we have designed and prepared aminoacid derivatives of some commonly used aminocyclitol antibiotics i.e. Netilmicin, Kanamycin. The attack on the molecules of these antibiotics by the modifying enzymes is shown on Figure 3.

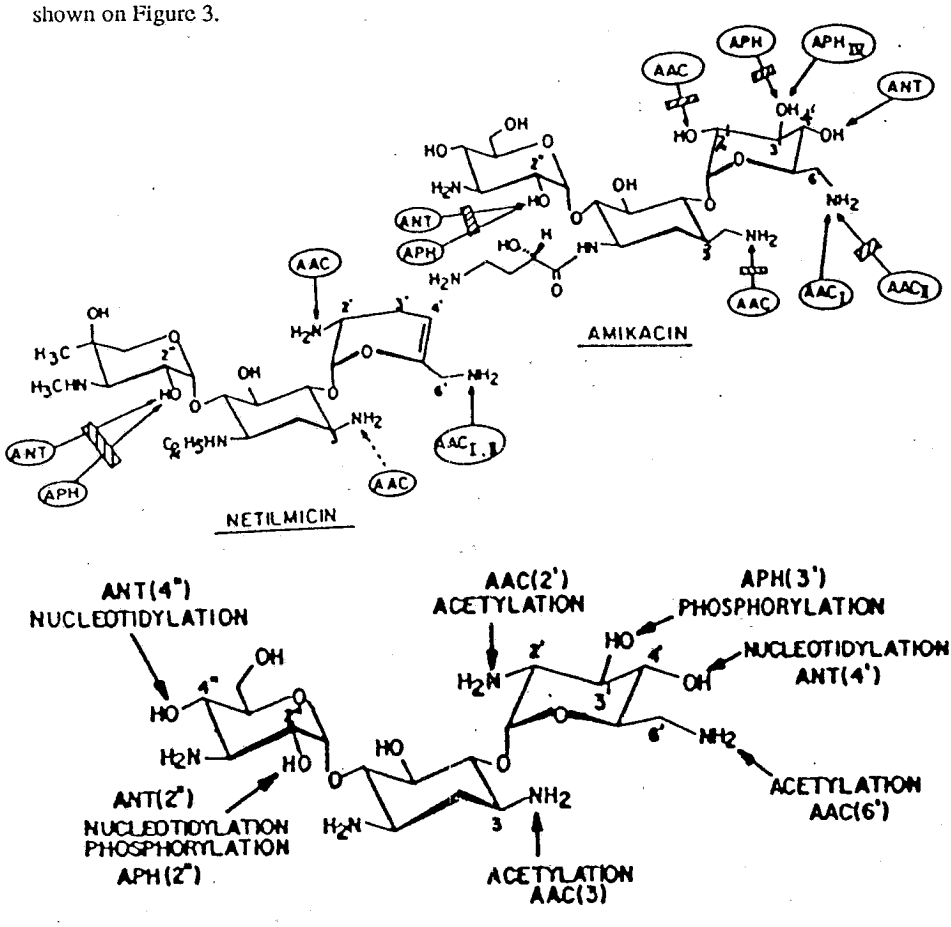
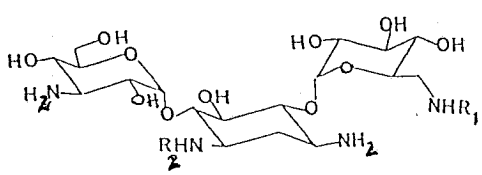


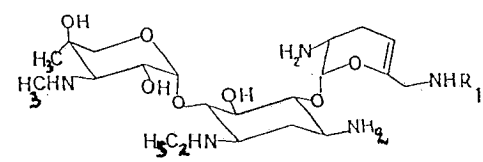
Figure 3. Aminoglycoside-modifying enzymes

Oto-and nephrotoxicity of aminoglycosidic antibiotics are related to the binding of these amino pseudopolysaccharides (in a protonated or neutral form) to the charged phospholipids and subsequent inhibition of the lysosomal phospholipases[6]. Preliminary work in our laboratories has shown that the 6' aminoacid derivatives of neamine were active[7]. This stimulated us to extend our work to the synthesis of amino acid ant peptide derivatives of kanamycin (1) and metilimicin (2). 6'-N or 1-N derivatives of 1 and 6'-N derivatives of 2 with common amino acids and peptide (L-Ala-OH, D-Ala-OH, Gly-OH, L-Leu-OH, L-Asp-OH, L-Ala-L-Ala-OH), were prepared using the active ester method for coupling. The selective formation of the amide bond at the 6'N position of aminoglycosides was performed in the presence of Cu^{2+} ions in one step[8]. The preferential complexation of vicinal hydroxy amino groups by Cu^{2+}, aimed us to synthesize the 1-N derivatives after selective protection of 6'-amino group of kanamycin A by tert-butoxycarbonyl group. The antibacterial properties are under investigation.



(1) : Kanamycin A (R_1,R_2=H)

1a : R_1 = Amino acid or peptide residue
L-Ala-OH,D-Ala-OH,Gly-OH,
L-Leu-OH,L-Asp-OH,
L-Ala-L-Ala-OH)
R_2 = H
1b : R_1 = H
R_2 = Amino acid or peptide residue
(L-Ala-OH,D-Ala-OH,Gly-OH,
L-Leu-OH,L-Asp-OH,
L-Ala-L-Ala-OH)



(2) : Netilmicin (R_1=H)

2a : R_1 = Amino acid or peptide residue
(L-Ala-OH,D-Ala-OH,Gly-OH,
L-Leu-OH,L-Asp-OH,
L-Ala-L-Ala-OH)

Scheme VII

The prepared compounds are expected to be not only invulnerable by the modifying enzymes, that is derive, but also less toxic since their structure and conformation[10] will prevent their interaction to the phospholipids of the proximal tubular cells in the kidney etc[11]. Since the high toxicity of the aminoglycosidic antibiotics is the main concern in their clinical use. The prepared compound, which are currently under biological evaluation, may be valuable against microbial infections.

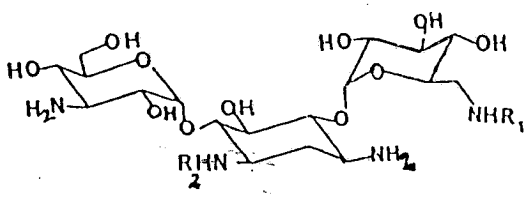
APPROACH B

The knowledge assembled on the mechanisms of uptake and distribution of drugs in cells and the mechanisms of cellular toxicities has prompted us to synthesize aminoglycosidic conjugates with β-lactams and quinolones with unusual pharmakokinetic properties[12]. These compounds are expected to have combined properties (and synergism) of the two major classes of antibiotics and thus wider spectrum of antibacterial activity.

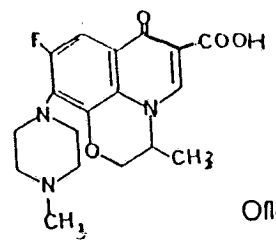
We have prepared two king of conjugates:

a) We have incorporated Kanamycin A (1) and netilmicin (2) as the aminoglycoside component and ofloxacin, norfloxacin or 6-amino-penicillanic acid as the quinolone or β-lactame component. Benzylocarbonyulamino-penicillanic acid and fluoroquinolones were couupled with 6'-amino group of aminoglycosides by an amide bond. The selective coupling was performed after treatment of aminoglycosides with DMSO solution of Cu^{2+}. The antibacterial properties are under investigation.

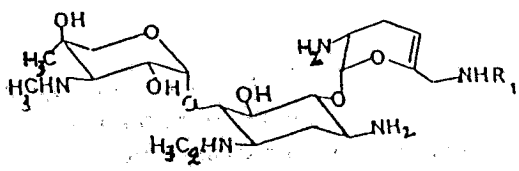
b) We have activated the C-6' or C-6" hydroxy or C-6" amino group of kanamycin A by converting them to activated carbonates with phosgene before coupling (or we have converted the same group to carboxylate functions) them with β-lactams or quinolones as depicted below.



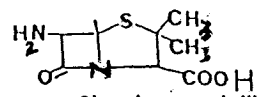
(1) : Kanamycin A (R_1,R_2=H)



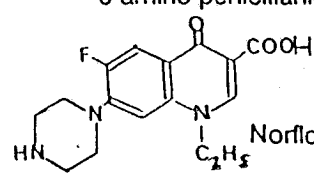
Ofloxacin



(2) : Netilmicin (R_1=H)



6'amino penicillanic acid



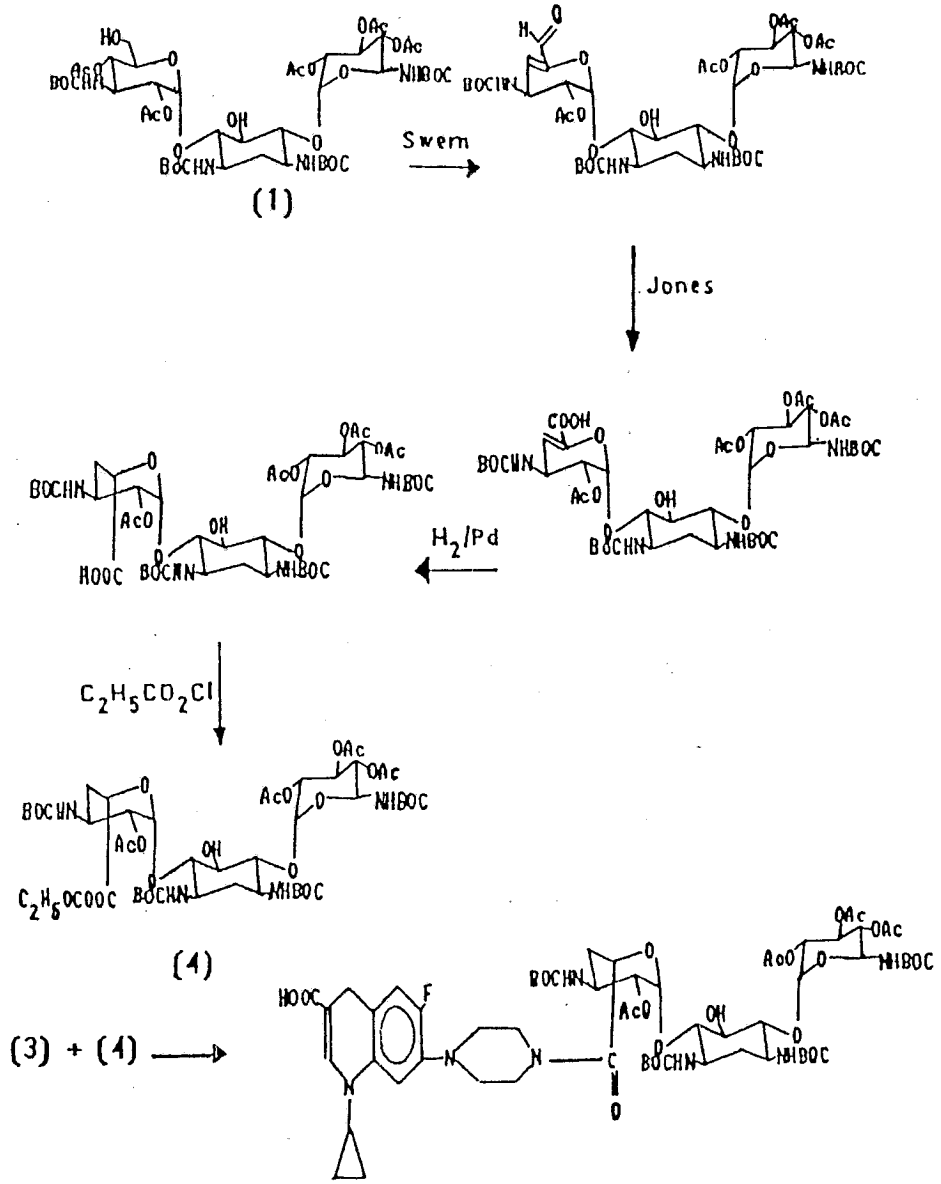
Norfloxacin

1a : R_1 = Fluoroquinolones (Ofloxacin, Norfloxacin) or β-lactam (6'-amino penicillanic acid) residue
R_2 = H

2a : R_1 = Fluoroquinolones (Ofloxacin, Norfloxacin) or β-lactam (6'-amino penicillanic acid) residue

9a

Scheme VIII



Scheme IX [5c]

Acknowledgment

I thank my collaborators and graduate fellows (chemists) at the Agricultural University of Athens for making this presentation possible, namely : Ioannis Grapsas, Ioannis Ierapetritis, Violetta Constantinou-Kokotou, Stamatia Kotretsou.

All of us we thank the National Drug Organization (ΕΟΦ) for the main financial support and its president till 1989 for encouraging mission oriented research. We also thank the Ministry of Industry Section of Research and Technological Development for a supplementary grant. We are grateful to the following Pharmaceutical Companies for donating chemicals or antibiotics:

GAP Pharmaceutical Co Athens Greece (chemicals)
Schering Pharmaceutical Corporatim Bloomfield N.J.(netilmicin)
UpJohn Pharmaceutical Co Kalamazoo Michigan (neomycin)
HELP Pharmaceutical Co, Ioannina-Greece (kanamycin)

Finally we thank Professor Kenneth L.Rinehart Jr of the University of Illinois for inspiring the initiation of our work on aminocyclitol antibiotics.

Περίληψις

Ημισυνθετικαί προσεγγίσεις διά αποτελεσματικώτερα αντιβιοτικά

Η φιλοσοφία και προσέγγισις δια την σύνθεσιν (ημισύθενσιν) καλυτέρων αντιβιοτικών εναντίον ανθεκτικών στελεχών των βακτηρίων που περιγράφεται εις το παραπάνω άρθρον εις την Αγγλικήν είναι η παρακάτω:

Α. Αλλαγή της χειρομορφίας εις τον άνθρακα του μορίου ο οποίος προσβάλλεται από τα ένζυμα μετατροπής (Modefying Enzymes). Περιγράφεται η αλλαγή της χειρομορφίας εις τον C-5' άνθρακα της Νεαμίνης δηλαδή η ημισύνθεσις της 5'-Επι-νεαμίνης.

Β. Κατά μίαν άλλη προσέγγισιν γίνονται δεσμεύσεις των χαρακτηριστικών ομάδων ειδικώτερα των αμινοομάδων που προσβάλλονται από ένζυμα μετατροπής με αμινοξέα (και διπεπτίδια) εις τα μόρια των αμινογλυκοζιτικών αντιβιοτικών. Έτσι επιδιώκεται η καλύτερη φαρμακοκινητικότης, ολιγότερη τοξικότης και καλύτερη δραστικότης (μη προσβολή από τα ένζυμα μετατροπής). Τέλος περιγράφεται η σύνθεσις μικτών αντιβιοτικών που προέρχονται από την σύζευξη β-λακτάμης και φθοροκινολονών με την Καναμυκίνη και Νετιλμικίνη με στόχον ευρύτερον φάσμα δράσεως και ολιγότερη τοξικότητα.

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CHIMIKA CHRONIKA, *NEW SERIES*

CONTENTS

April - June 1993 Volume 22 No 2

This year visit Macedonia

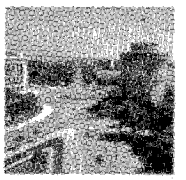
Macedonia

For 4,000 years* steeped in the history of Greece

Statue of Aristotle. Stagira.

Aristotle, the tutor of Alexander the Great, was born in Stagira in Macedonia in 384 BC. Together with Plato, he is regarded as one of the greatest philosophers the world has known. Aristotle was a true academic, concerned with Physics, Astronomy, Rhetoric, Literature, Political Science and History. His teachings laid the foundation for modern scientific thought.

The White Tower of Thessaloniki.

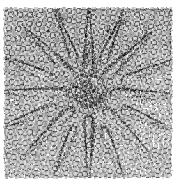


Thessaloniki, the heart of Macedonia, is a modern city with 1,000,000 inhabitants. It is strategically located at the crossroads of Europe with Asia. Having spread the Word at Philippi, the Apostle Paul continued his teachings in Thessaloniki. Its important monuments from antiquity and byzantium up to the present, provide testimony to the role that the city has played as the second capital of Hellenism.

The Bust of Alexander the Great. Acropolis Museum. Athens.

Alexander was born in 356 BC in Pella, Macedonia, established by his father Philip II, as the centre of Hellenism. Nurtured on the thoughts of his tutor, Aristotle, he rose to fame as a brilliant military leader. He influenced the course of history, rightfully earning his title as Alexander the Great. In 335 BC he became Commander in Chief of all the Greeks. By the time of his death in 323 BC he had created an enormous empire, stretching from the shores of the Adriatic to India, and from the Caucasus Mountains to Egypt. He spread the Greek spirit far and wide among nations who worshipped him as a god.

Symbol of the Greek Macedonian Dynasty from the tomb of Philip II. Archaeological Museum. Thessaloniki.



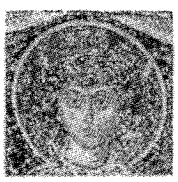
This 16 pointed star of Vergina was uncovered during the archaeological excavations at Vergina. This symbol of the Greek Macedonian Dynasty decorated the golden tomb of Philip II. The Star of Vergina, extracted from the soil of Macedonia, has since become the symbol of Hellenism.

4.000 years: Post-Mycenaean ceramic relics found in Assiros and Mycenaean swords found in Grevena date back 4.000 years, evidence of Macedonia's role at the vortex of Greek history. Even in mythology Macedon, mythical founder of the Macedonian race, is the son of Aeolos (god of the winds). Throughout the years Macedonia contributed to the fountain of knowledge of the Ancient Greeks. In the 5th century BC Demokritos, father of Atomic Theory, lived and worked in Avdira.*

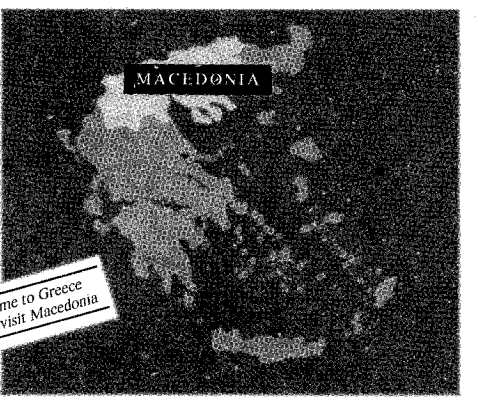
The OlympianAphrodite (3rd Century BC). Museum of Dion.

This statue of Aphrodite came to light during archaeological digs at the ancient sacred city of Dion. Dion, at the foot of Mt Olympus, was the most important spiritual site for the Northern Greeks, playing the same role in their lives as that of the oracle at Delphi.

St Dimitrios. detail of 7th Century Mosaic. Church of St. Dimitrios. Thessaloniki.



St Dimitrios, Protector of the city of Thessaloniki, was martyred in 305 AD defending Christianity. He is regarded as the Patron Saint of Thessaloniki and its saviour during difficult moments.



GREECE
Chosen by the Gods