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REVIEW

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CHEMISTRY, BIOCHEMISTRY AND NUTRITIONAL ASPECTS OF NATURAL PHOSPHONO-COMPOUNDS. I. GLYCERO-PHOSPHONOLIPIDS

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Summary

To date the research on the existence of natural aminoalkyl phosphonic acids has proceeded in 3 directions. I. Research on the existence and structural characterization of C-P compounds in living organisms. II. Metabolism, biosynthesis and transport of C-P compounds. III. Function of C-P compounds i) as antibiotics ii) as pesticides iii) on membrane potentials.

This is the first review of a series of reviews related to all the above topics, describing present knowledge on the naturally occuring phosphono-analogs of glycerolipids including the natural aminoalkyl phosphonic acids and some general metabolic aspects of phosphono-coumpounds.

The presence of glycero-phosphono-lipids has been reported mainly in the following organisms: 1) Higher mammals (in human tissues as brain, heart, muscle, liver etc., also in bovine bile and milk, 2) Coelenterata 3) Mollusca 4) Protozoa and 5) Mycobacteria and Fucacae.

Key words : Aminoalkyl phosphonic acids, 2-Aminoethyl phosphonate, Glycerophosphonolipids, Coelenterata, Mollusca, Protozoa, Mycobacteria and Fucacae, N,N,N, Trimethyl aminoethyl phosphonate.

Abbreviations and Terminology : AEP 2-Aminoethylphosphonate; DG-AEP Diglyceride-AEP; TMAEP N,N,N, Trimethyl-AEP; CTP Cytidyl-triphosphate; CDP Cytidyl-diphosphate; CMP Cytidyl-monophosphate; p_nAla β-Phosphono-alanine; PE Phosphatidyl ethanolamine; PC Phosphatidyl choline.

Introduction

Phospholipids are essential components of membrane structures. There may be a similar role for natural phosphonolipids. Since the C-P bond is highly resistant to

hydrolysis by acids or bases, and to the action of snake venom phosphodiesterases [1] an important biological advantage might be conferred by the presence of phosphonolipids in membranes. In the gastrovascular cavity of sea anemones, into which many hydrolytic enzymes are released, cell membranes may benefit from this stability. The major phosphonate is 2-aminoethyl-phosphonate (AEP), an analog of ethanolaminephosphate.

The presence of AEP in proteolytic enzymes from sea anemones suggests that substances with C-P bonds may provide groups similar to phosphate but which are stable to the action of other enzymes in the gastrovascular cavity. Similar reasoning may be applicable in the case of surface structures of ciliates which are found in the phosphatase-rich environment of the rumen.

Since there is ready incorporation of dietary amino-phosphonic acids into lipids in mammalian organisms, the determination of the sites and of the extent of deposition of these substances, as well as the possible effects on membrane functions will be of considerable interest. This may be of particular importance in cases where the chief dietary sources are marine or dairy products.

It is of particular interest to marine biology that C-P compounds are major constituents in three phyla and that they are synthesized by phytoplankton, the base of the food chains in the ocean. This observation forces a reevaluation of the phosphorus cycle in the sea. Since the utilization of AEP by bacteria may be inhibited by orthophosphate [2], the fraction of the total phosphorus which becomes incorporated into C-P compounds only slowly may be returned to the cycle.

To date, naturally occurring lipids and proteins with a direct carbon to phosphorus bond have been discovered in a wide variety of organisms ranging from bacteria to mammals.

The research on the existence and destiny of natural aminoalkyl phosphonic acids has proceeded in 3 directions.

I. Research on the existence and structural characterization of C-P compounds in living organisms
   - I.1. C-P compounds as components of glycerolipids.
   - I.2. C-P compounds as components of sphingolipids.
   - I.3. C-P compounds associated with proteins.

II. Biosynthesis, Metabolism and Transport of C-P compounds.

III. Function of C-P compounds.
   - III.1. Studies on phosphonic acid Antibiotics or Drugs as inhibitors of bacteria cell wall synthesis.
   - III.2. Studies on the use of synthetic phosphonic analogs of naturally occurring substances as herbicides or pesticides.
   - III.3. Influence of C-P compounds on membrane potentials.

We have undertaken the task to review present knowledge related to all the above topics in a series of reviews following the mentioned tentative classification. This is the first review of the series describing present knowledge on the naturally occurring phosphono analogs of glycerolipids (subclass I.1. above), including the natural aminoalkyl phosphonic acids and some general metabolic aspects of

phosphono compounds. The latter are unavoidably connected with all kinds of research in the field of natural phosphono compounds.

Natural occurrence of glycerophosphonolipids

The full extent of the distribution of glycero-phosphonocompounds in nature remains to be established. To date the presence of glycero-phosphonolipids has been reported mainly in the following organisms :

Higher Mammals

Alhadeff et al.[3] have discovered that AEP is present in both, lipid and proteinaceous fractions of human brain of a 38 year-old caucasian male who died as a result of a crushing chest injury. The brain was removed, frozen and stored for 3 years prior to extraction.

One year later Alhadeff et al. have discovered AEP in 4 human tissues[4]. Its concentration was higher in heart and muscle than in liver or brain. The distribution of AEP between polar and non polar fractions was examined and found considerably different. The higher concentration was found in the protein rich fraction.

Also it was discovered that AEP is present in both, lipid and proteinaceous fractions of goat liver[5] and of beef brain[6]. Hasegawa et al.[7] have found a high accumulation of AEP in bovine liver lipids, mainly as DG- AEP (the phosphonate analog of phosphatidylethanolamine). Namely, in the phospholipid fraction, 96% of total phosphonolipids was detected as DG-AEP and only 2,6% was found as the phosphonate analog of phosphatidyl-choline. The presence of TMAEP in the acid hydrolysates of the lipid fraction (and of the nonlipid fraction) was demonstrated by T.L.C. The authors suggested that AEP may also exist as the phosphonate analogs of ceramide phosphorylethanolamine, (sphingoethanolamine) and sphingomyelin. Significant differences in the distribution of AEP were observed among the non lipid fractions of mitochondria, microsomes and soluble cytoplasm.

AEP was also isolated from bovine bile, where it may exist as a phosphonic acid analog of taurocholic acid. On another hand, bovine gall bladder bile was found to contain three species of phosphonolipids DG-AEP, DG-TMAEP and ceramide-AEP[9]. These results suggest that AEP originating from rumen protozoa may also exist in many tissues, secretions and excreta of all kinds of ruminants, indicating a need for an investigation of the physiological role of AEP in ruminants with special interest the enterohepatic circulation[8].

Coelenterata

Several different sea anemones as well as corals, have been examined. The results of early investigations of lipids of the sea anemones were somewhat conflicting. Kittredge, Roberts and Simonsen [10] have found AEP bound to glycerol. They applied the 70% ethanol extract of *Anthopleura elegantissima* to a mixed bed ion exchange resin and isolated the free glycerol ester of the AEP. The compound

was not isolated in sufficient quantity for confirmation of its structure as a glyceryl-AEP and it was not known at that time which hydroxyl of glycerol is bonded to AEP. But one year later (1963) in another report from workers of the same laboratory [11] it was reported that the lipids extracted from the same sea anemone with Chloroform methanol were fractioned, with quite divergent results from those reported earlier. Namely a sphingolipid containing AEP was isolated and shown to be ceramide AEP.

In the rest of coelenterata studied so far, i.e. in the anemones *Metridium dianthus* [12] and *Taelia felind* [13], and in the coral *Zoanthus sociatus* [14], the 70% ethanol extract was found to contain free AEP accompanied by a sphingolipid containing AEP.

Mollusca

The marine bivalbe *Mytilus edulis* (blue Mussel) or *Venus mercenaria* [13] was found to contain AEP (5g per 53-64 g fresh animals), and most of the phosphonate compounds could be extracted by aqueous ethanol.

Fresh-water bivalves were studied and found to contain AEP [13,15], (free and in the form of phosphonosphingolipids); the animals were *Corbicula sandai, C. Japonica, Unio biwal, Inversidens hirasei, Anodonta lauta restata* and *Cristaria phicata.* Similar findings were reported for gastropods [16].

Terrestrial Molluscs [17]: In the garden slugs *Lehmannia poirieri* and *Limus flavus* about 16% of the phosphonate is in the free, acid-extractable state, some 23% is bound to chloroform-soluble lipid, and the remainder is in the lipid free residue of the acid extract with lipid solvent. On the other hand in the garden snail *Helix aspersa* about 51% of the total phosphonate is acid soluble, some 32% bound to lipid and 17% to the «residual» material, but glycerolipids were not detected. The biosynthetic pattern suggests that in both terrestrial molluscs the soluble (free) AEP is the precursor of the bound form. This is in contrast with the biosynthetic pattern in Tetrahymena, where phosphonoenolpyruvate seems to be the precursor of the carbon atoms of bound AEP (see below).

Protozoa

In *Tetrahymena pyriformis* the phosphonic acids are present in free form and bound to lipid. The presence of glyceryl-AEP had been demonstrated in the products of alkaline hydrolysis of Tetrahymena lipids and a pure cephalin fraction, consisting of phosphatidylethanolamine and its phosphonic analog in a molecular ratio of approx. 13:1 had been isolated from this species [18].

The presence in Tetrahymena of a glyceryl monoether phosphatidyl-AEP was established by Thompson [28], who found that Tetrahymena membranes contain two major phospholipid fractions tentatively named PE and PC fractions. The choline lipids (PC fraction) are rich in glyceryl ether analogs of lecithin, and the PE fraction is in fact a mixture of four types. (PE, DG-AEP, and the glyceryl ether of both). Thompson studied the formation of these lipids from a number of radio-active precursors and the most active ones were palmitic and acetic acid, and chimyl alcohol [28].

Later Chacko and Hanahan[29] synthesized the monoether analog of DG-AEP and confirmed the presence of both, the diacyl and alkyl akyl analogs in Tetrahymena.

According to more accurate studies[45,46] the «PE-fraction» of Tetrahymena contains 30-70% phosphono analogs. In the latter the percentage of the glyceryl ether form varies between 30%[45] and 55%[46], whereas the phosphoanalogs consists 100% of the diester form.

Later Sugita and Hori[31], isolated DG-AEP from *Tetrahymena pyriformis.* The molar ratio of phosphorus to fatty acid in the isolated lipid was 1,00:1,97. Gas liquid chromatography showed mainly palmitic (13,2%), stearic (52,7%), and oleic (22,0%). The next year, 1972, Tamari[32] studied the intracellular distribution of AEP, in *T. Pyriformis* (GL) and found that the mitochondria contain 19% of AEP.

In addition to AEP, another phosphonic acid, 3-phosphonoalanine (P_nAla) was found in Tetrahymena, but this was absent from the lipids of Tetrahymena; it was present in the free form and in the insoluble residue[30].

In 1970 Kennedy and Thompson[35] have found that approximately 60% of the phospholipids from the membrane sheath of *T. pyriformis* cilia contain AEP in more than twice the concentration found in total cellular lipids. The resistance of these lipids to hydrolytic enzymes suggests that they increase the stability of the surface membranes.

Rhoads and Kaneshiro[43] characterized phosphonolipids from *Paramecium tetraurelia* cells and cilia (strains 51s and d_4 95). In whole cell lipids they identified the following classes of phospholipids: the 1-alkyl-2-acyl-glyceryl and the 1,2-diacylglyceryl forms of AEP, phosphoryl ethanolamine and phosphorylcholine, cardiolipin, ceramide- AEP, 5 other spingolipids, phosphatidylserine, phosphatidylinositol, and lyso derivatives of the major glycerophospholipids. Cilia lipids were rich in ether lipids, phosphonolipids and sphingolipids. Total lipids per cell decreased with culture age, changes in the neutral lipid fraction accounting for the major decrease. Phospholipid class distributions changed also with culture age. Namely the PE and PC contents of the cells was decreased, while the DG-AEP content remained relatively constant and, therefore, the ratio of phosphonolipids to total phospholipids was increased. All species of fatty acids observed in total lipids from cells and cilia were also present in the glycerophospholipids. Total lipids from cilia contained a greater percentage of polyunsaturated fatty acid than those from whole cells. Whole cells and cilia glycero-phosphonolipids contained up to 93% eicosatetraenoic plus eicosapentaenoic fatty acids. The enrichment of phosphonolipids in cilia accounted for most of the polyunsaturated fatty acid enrichment of cilia lipids. The fatty acid composition of all the major glycerophospholipid classes of whole cells were changed dramatically with culture age, while only small changes occurred in cilia glycerophospholipid fatty acids.

One year later (1980) the same group[44] examine the fatty acid positional distributions and the fatty acid compositions of diacyl and alkyl acyl analogs of the 3 major glycerophospholipids of Paramecium. PC, PE and DG-AEP were separated into diacyl and alkyl acyl species by T.L.C. after phospholipace C digestion and acetylation. As in Tetrahymena, PC and DG-AEP of Paramecium

were predominantly in the alkyl acyl form, whereas PE was predominantly in the diacyl form. Complete and efficient hydrolyses of all 3 major glycerophospholipids were accomplished with phospholipase A_2 from porcine pancreas in the presence of Sodium tetraborate in order to prevent acyl migration. Saturated acids were found predominantly at C-1 of diacyl phospholipids, while polyunsaturated fatty acids were mainly at C-2, particularly in the alkyl acyl species. An exception was γ-linolenate, which was a major acid found esterified to C-1 in all 3 diacyl phospholipids. Identification of this acid at that position supports the idea that in some ciliates there may be an acyltransferase, specific for γ-linolenate, that esterifies this acid to the glycerophospholipids.

Mycobacteria and Fucacae

In 1970 Sarma et al.[41] have shown the occurrence of phosphonolipids in *Mycobacterium tuberculosis* $H_{37}R_V$, *M. phlei* (N.C.T.C.) and *Mycobacterium 607* (A.T.C.C.). The cells were grown on modified medium, and then were harvested and the lipids extracted by the method of Folch et al. The lipids were chromatographed on silicic acid according to the method of Rouser[11]. The phospholipids were dissolved in chloroform and stored at -20 °C. Of the 3 strains studied, *M. phlei* contains the smallest amount of phosphatides while the virulent pathogenic *M. tuberculosis* $H_{37}R_v$ contains a relatively high percentage of them. The other saprophytic strain, 607, contains a higher amount of phospholipids as compared to *M. phlei,* but less than that of *M. tuberculosis* $H_{37}R_v$. On the contrary, while in *M. tuberculosis* H_rR_v the phosphonolipid content is very low. *Mycobacterium 607* and *M. phlei* contain relatively higher amounts of phosphonolipids. Liem and Laur[42] studied the structure, percentage and composition of sulphuryl, sulfonyl and phosphonyl glycosyl ester diglycerides in three Fucacae *(Pelvetia canaliculata, Fucus vesiculosus, F. Serratus).* They found that the mono and polyester glycosyl sulfates or phosphate diglycerides account for a group of polar lipids which is found in large amounts. These polar lipids were shown to have complex structures, most of them unknown in the present nomenclature.

Aspects of AEP and glycerophosphonolipid metabolism

Todate it is generally accepted that AEP is formed from phosphoenolpyruvate[36] but there is some disagreement over the details of the biosynthetic pathway. Horiguchi[47,48,49] has demonstrated a soluble pathway, in which doubly labeled phosphoenolpyruvate is converted to free AEP having the same $^{14}C/^{32}P$ ratio as the starting material, through phosphonopyruvate and phosphonoacetaldehyde as intermediates. Alternatively, phosphoenolpyruvate reacts with phosphatidyl-choline, using the phosphatidic acid portion of the lipid to give lipid bound intermediates and aminoethylphosphonolipid directly[20,50,28,51].

As evidence supporting this latter pathway has been considered the observation that when *Tetrahymena* is grown in the presence of ^{32}P, the specific activity of the lipid-bound AEP after 1 h is considerably greater than that of the protein-bound or

even of the soluble AEP, and only after much longer times do these latter specific activities approach that of the lipid-bound AEP[20,50]. In addition, when Tetrahymena is labeled with either palmitate or chimyl alcohol, the diglyceride backbone of phosphatidylcholine is apparently transferred to form aminoethylphosphonolipid[28,51].

In 1966 Liang and Rosenberg[18] had presented evidence for the existence of a pathway in Tetrahymena, whereby DG-AEP can be synthesized. This pathway does not differ from that described for the synthesis of phosphatidyl choline or ethanolamine[19], except that the phosphonate, rather than the ester phosphate of the base is utilized. The reactions involved can be represented as :

$$\text{CTP} + \text{AEP} \rightarrow \text{CMP-AEP} + \text{pyrophosphate}$$
$$\text{CMP} - \text{AEP} + \text{diglyceride} \rightarrow \text{«AEP-Cephalin»} + \text{CMP}$$

While it was later realized[18] that, taken separately, the various pieces of evidence could not provide absolute proof, they were mutually supporting in that they comprised a complete pathway with a well established precedent. However, one important aspect of the above results which should not be overlooked is that the above pathway may serve merely as a salvage mechanism for the recovery of any free AEP that may arise as a result of the breakdown of some of the lipids or of other insoluble components[20]. In addition, it was recognized that the described pathway is not at all specific for AEP. It is likely that AEP is simply used, as ethanolamine phosphate would be, by the usual phospholipids- synthesizing system, which in other species has been shown to lack specificity with respect to the base[21]. Although AEP has not been tested in such experiments, there were indications that the mammalian systems cannot reject AEP as a source of phospholipid bases. Such indications were provided by reports on the occurrence of AEP-containing lipids in ruminants which harbor protozoa[22,23] and by the findings that administered AEP is incorporated into the brain lipids of the rat[24].

In contrast, it was shown experimentally, that a chicken kidney microsomal fraction which synthesizes phospholipid and serine-ethanolamine phosphodiester[25], could synthesize the respective phosphonate-containing lipid and diester analogs when incubated with CMP-AEP rather than with CDP-ethanolamine as substrate. However it was still necessary to isolate the pure lipid without the contaminating PE.

The thin-layer chromatographic separation of phosphono and phospho-lipid analogs was later achieved by Kapoula[26] with the use of several novel solvent combinations, i.e. mixtures of chloroform with high proportions of 85-92% acetic acid, to which up to 5% methanol may be added when separation of phosphatidyl serine from lysophosphatidyl ethanolamine, phosphatidyl inositol and basic phospholipids is desirable[26].

Smith and Law[30] have provided the first evidence for the *in vivo* uptake by Tetrahymena of AEP and for its incorporation into DG-AEP, as well as for the conversion of Pn Ala to AEP, although these experiments do not indicate whether this conversion occurs at the phospholipid level or in the free form. No labeled

P_nAla was found in phospholipid even though DG-P_nAla had been suggested as an intermediate in DG-AEP biosynthesis. The authors suggested that the phosphonic acid metabolism perhaps takes place in a specialized compartment within the cell and that there is not general exchange with cellular pools during the biosynthesis of phosphonolipids. When phosphonic acids are supplied from outside the cell, they may be transported to this special location for both incorporation into lipids and for degradation in the case of P_nAla.

La Nauze and Rosenberg[33] have shown that extracts of *Bacillus cereus* can convert AEP into phosphono-acetaldehyde and inorganic phosphate. Also Robert et al.[34] have shown that cell-free preparations of *Tetrahymena* catalyze the breakdown of both AEP and P_nAla.

Generally with AEP addition the amount of the phosphonolipid is doubled at the expense of phosphatidyl ethanolamine[39]. However when the cells are grown with 3-aminopropylphosphonate, which is isosteric with ethanolamine phosphate, it replaces the latter in phosphatidylethanolamine without altering the phospholipid composition[40], e.g. in both these experiments the level of the phosphatidylcholine was not affected. These results suggested that the pathways for the biosynthesis of PE and DG-AEP from ethanolamine phosphate and AEP, through CDP-ethanolamine and CMP-AEP respectively, utilize different enzymes. However, a control mechanism is apparently present which provides a minimum level of phosphonolipid in the cell. In 1972 Bjerre had shown that TMAEP is incorporated into the phosphatidyl choline fraction of rat liver both in vivo and in vitro[38] although the effect of the overall phospholipid composition was not determined.

In 1981 Smith and Giegel[36] have used the phosphonic acid analog of choline phosphate, TMAEP, in Tetrahymena to determine the relationship of phosphatidyl choline biosynthetic pathways to those of the other two phospholipids, despite that TMAEP does not occur in *Tetrahymena*[30,37].

The results were that, up to 60% of the choline phosphate in PC was replaced by the phosphonic acid. Also there was an increase in the relative amount of quaternary ammonium-containing lipid (PC plus TMAEP-lipid) at the expense of PE and no effect, of the TMAEP, on either AEP-lipid levels or on de novo AEP-synthesis.

The incorporation of TMAEP into the «phosphatidylcholine», in place of choline phosphate, might be expected to affect AEP biosynthesis if AEP is formed at the lipid level[36].

Περίληψη

Περί των φυσικών φωσφονικών ενώσεων από την πλευρά της Χημείας της Βιοχημείας και της Διατροφής.
I. Γλυκεροφωσφονολιποειδή.

Συνολικά σήμερα η έρευνα για την ύπαρξη και σημασία των φυσικών

φωσφόνο-ενώσεων, δηλ. των ενώσεων που φέρνουν στο μόριο τους τον σπάνιο και εξαιρετικά ανθεκτικό δεσμό άνθρακα φωσφόρου (C-P), έχει αναπτυχθεί σε 3 κατευθύνσεις :

I. Απομόνωση από ζωϊκούς ιστούς όπου ευρίσκονται συνδεδεμένες με γλυκερολιποειδή ή σφιγγολιποειδή ή πρωτεΐνες.

II. Μεταβολισμός-βιοσύνθεση και μεταφορά των φωσφόνο-ενώσεων.

III. Δράση των φωσφόνο-ενώσεων.

(i) ως συστατικά των αντιβιοτικών, διότι αναστέλουν την ανάπτυξη των βακτηριδίων (ii) ως δραστικό συστατικό σε ζιζανιοκτόνα (iii) στη μεταβολή της διαφοράς δυναμικού μέσα στις κυτταρικές μεμβράνες.

Η παρούσα εργασία είναι το πρώτο άρθρο ανασκόπησης από μια σειρά άρθρων που θα καλύψουν όλο το πεδίο, και αναφέρεται στα φωσφόνο-γλυκερολιποειδή καθώς και σε μερικές γενικές απόψεις για το μεταβολισμό τους. Μέχρι σήμερα συναντώνται στα ανώτερα θηλαστικά, όπως άνθρωπος, βόδι, κατσίκα κ.λ.π. καθώς και στο γάλα. Επίσης στα βλεφαριδοφόρα πρωτόζωα, στα βακτήρια καθώς και σε μαλάκια και κνιδόζωα.

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KINETIC STUDY OF THE OXIDATION OF RING MONOSUBSTITUTED AROYLHYDRAZINES BY COPPER(II) CHLORIDE. APPLICATION OF THE HAMMETT EQUATION

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Summary

The kinetics of the oxidation of halogeno, nitro and methoxy ring monosubstituted aroylhydrazines by copper(II) chloride was studied spectrophotometrically at temperatures between 25 and 60 ^{0}C and pH range 4.40 to 5.60.

The main reaction is of first order for both the aroylhydrazine and for the copper(II) chloride. The second order reaction rate is inversely dependent on the hydrogen ion concentration. The energy of activation ranges between 79.5 and 110.0 kJ.mol^{-1} whereas the values for the p-substituted aroylhydrazines are almost the same varing between 91.6 and 96.2 kJ.mol^{-1}. The application of the Hammett equation showed that the reaction rate exhibits a small dependence on the substituent ($\rho = -0.10$) and that the m-F and p-NO_2 substituted aroylhydrazines deviate appreciably. Generally, the reaction seems to be favored by substituents acting as electron donors. In conclusion, the substituents act rather upon the ability of the aroylhydrazines in forming coordination compounds with the Cu(II) ions and influences the electron transfer rate negligibly.

Key words : redox system, ring monosubstituted aroylhydrazines, reaction rate, inductive effect, resonance effect, Hammett equation.

Abbreviations : XBH : ring monosubstituted aroylhydrazines (X = F, Cl, Br, I, NO_2 and OCH_3 in o-, m- or p- position.

Introduction

The redox system of the benzoylhydrazine and the copper(II) chloride has been investigated in a previous paper.[1]. Further insight on the mechanism of the reaction was considered as efficient by introducing substituent groups to the benzene ring[2]. In the present paper we report the influence of the inductive and the resonance effects of eighteen substituents upon the kinetics of the oxidation of the arising ring monosubstituted aroylhydrazines by copper(II) chloride. The aroylhydrazines have

the general formula: $X\text{-}C_6H_4C(O)NHNH_2$ where X indicates F, Cl, Br, I, NO_2 and OCH_3 in o-, m- or p-position.

Experimental

Reagents and instruments

The aroylhydrazines were prepared from the corresponding aromatic acids by adding the appropriate amount of hydrazine hydrate to their ethylesters and purified by recrystallization from warm water[3,4]. The solutions of the aroylhydrazines were prepared in triple distilled water and were deairated by continuous argon bubbling. The copper(II) chloride solutions were prepared from Merck pro analysi $CuCl_2 \cdot 2H_2O$, recrystallized from water. The copper(II) solutions were checked iodometrically. The potassium chloride solution for constant ionic strength was prepared from Merck pro analysi KCl. The buffer solutions (pH range 3.8-5.6) were prepared by mixing the appropriate quantities of CH_3COOH and KOH solutions[5]. The CH_3COOH became free of reductive agents by distillation in the presence of $KMnO_4$. The pH measurements were carried out in a Beckman Research pH-meter.

The rate of the reaction was followed with a Carl Zeiss PMQ II spectrophotometer equipped with a temperature standardizing system. The measurements of optical density vs time at constant wave lengths were scanned between 260 and 240 nm where is the $\pi^* \leftarrow \pi$ excitation state of the system[6]. Measurements were also carried out by using a stopped flow spectrophotometer equipped with a Durrum logarithmic amplifier Model D 131. The reaction rate measurements were taken in the temperature range of 25-60 ^0C.

The identification and the determination or the reaction products were carried out in solutions of initial concentrations $1 \times 10^{-2} - 1 \times 10^{-3}$ M for the $CuCl_2$ and $1 \times 10^{-3} - 1 \times 10^{-4}$ M for the aroylhydrazines. The concentrations of the aroylhydrazines used for the determination of the rate constants were $4 \times 10^{-5} - 8 \times 10^{-5}$ whereas the concentrations of $CuCl_2$ were 5 to 10 times higher. For comparable results the measurements of optical density vs time were taken in the pH range of 4.70-5.30 at a constant ionic strength of 1.25×10^{-2} M.

The standard solutions of the aroylhydrazines were practically unchanged during the experiments[7].

Preparation of aroylhydrazines

The prepared aroylhydrazines and the corresponding m.p. are as follows* :

o-F:72-73 (70)^0C[8]; m-F:138.5-139 ^0C[4]; p-F:159.5-161 ^0C[4]; o-Cl:116-117 (109-110)^0C[9]: m-Cl:156-157 (154-156)^0C[10]: p-Cl:162.5-164 (163)^0C[11]; o-Br:148.5-150 (153)^0C[12]; m-Br:153-155 (151)^0C[13];p-Br:165 (164)^0C[14]; o-I: 194-195^0C[4]; m-I:

* M.p. in parentheses are from bibliography.

145-146 (140-142)^{0}C[15]; p-I:164-165 (168-169)^{0}C[16]; o-NO_2:119-121 (123)^{0}C[17]; m-NO_2:150.5-152 (152)^{0}C[18]; p-NO_2:208-210 (210)^{0}C[19]; o-OCH_3:92.5-94^{0}C; m-OCH_3:89-91^{0}C[20]; p-OCH_3:133.5-135 (136)^{0}C[21].

The m.p. were taken with a Büchi, Apparat nach Dr Tottoli, Pat. 320,338 equipped with 300^{0}C thermometer of ± 1 ^{0}C accuracy.

Results and discussion

Stoichiometry of the reaction

The identity of the reaction products as well as their molar ratio showed that the main reaction follows the one electron path :

$$X\text{-}C_6H_4\text{-}CONHNH_2 + CuCl_2 + H_2O \rightarrow$$

$$\rightarrow X\text{-}C_6H_4\text{-}COOH + \frac{1}{2} N_2 + NH_4Cl + CuCl \quad (1)$$

It is also evident that, to a very small extent, the reaction follows the four electron mechanism :

$$X\text{-}C_6H_4\text{-}CONHNH_2 + 4CuCl_2 + H_2O \rightarrow X\text{-}C_6H_4\text{-}COOH + N_2 + 4CuCl + 4HCl \quad (2)$$

The study of the reaction products at higher temperatures (80-100 °C) showed an increase of the ratio N_2:NH_3. On the other hand, the concentration of the produced Cu(I) tends towards the estimated value according to equation (2). This is in accordance to the mechanism of the oxidation of hydrazine as given by Cahn and Powell[22].

Reaction (2) was also found to have taken place during oxidation of aroylhydrazines in the presence of oxygen. This reaction is catalysed by Cu(II) and Cu(I) salts but no quantitative change of the Cu(II) to Cu(I) ions is reported[23].

Kinetics of the reaction

The application of the isolation method[24] showed that the main reaction is of the first order for either the aroylhydrazine or the copper(II) chloride. Besides, the reaction rate is inversibly dependent on the H^+ concentration in the pH range of 4.4-5.6. At higher pH values the reaction rate increases irregularly while the hydrolyzation products of the Cu(II) ions become more complicated. At pH values lower than 4.4, the reaction rate decreases quickly and near a pH value of 4.0 no reaction kinetics are assentially observed. The overall rate low as well as the related equations are those of the benzoylhydrazine.

Calculations of the rate constants

Calculations of the $k/mol^{-1}ls^{-1}$ values are achieved by application of the second

order reaction integrated rate expression[1], where $k = k' + k_\alpha[H^+]^{-1}$, k' is the rate constant of the acid independent path and k_α is the rate constant of the acid dependent path. Experimentally, no evidence has been found for an acid dependent path.

The k_α values of the benzoylhydrazine are $(3.1 \pm 0.4) \times 10^{-5}$ s^{-1} at 60°C, $(12.4 \pm 0.6) \times 10^{-5} s^{-1}$ at 50°C, $(5.2 \pm 0.3) \times 10^{-5} s^{-1}$ at 40°C and $(1.1 \pm 0.1) \times 10^{-5}$ s^{-1} at 25°C. The assumed activation energy is 79.9 ± 1.7 kJ.mol^{-1}. The determined k_α values of the oxidation reaction of the aroylhydrazines mentioned and the corresponding E_α values are given in the Table I.

TABLE I : The reaction rate constants k_α/s^{-1} of the oxidation of aroylhydrazines and the E_α values of the reaction. Temperature range 25-60°C, $I = 1.25 \times 10^{-2}$ M.

Aroylhydrazine	Temp. °C	o- $k_\alpha.10^5$ s^{-1}	o- E_α kJ.mol^{-1}	m- $k_\alpha.10^5$ s^{-1}	m- E_α kJ.mol^{-1}	p- $k_\alpha.10^5$ s^{-1}	p- E_α kJ.mol^{-1}
F-BH	50	11.2	100.4	28.2	90.4	16.1	96.2
	40	3.3		9.7		5.1	
	25	0.5		1.7		0.8	
Cl-BH	50	34.5	93.3	17.6	109.2	15.9	95.8
	40	11.2		4.6		5.1	
	25	1.9		0.6		0.8	
Br-BH	50	*		17.8	109.2	17.3	94.6
	40			4.8		5.7	
	25			0.6		0.9	
I-BH	60	*		34.8	86.6	48.1	91.6
	50			13.2		17.3	
	40			4.7		5.8	
	25			0.9		1.0	
O_2N-BH	60	17.6	110.0	45.4	96.7	24.8	94.6
	50	5.0		15.4		8.5	
	40	1.4		4.9		2.8	
CH_3	60	16.5	92.5	35.1	79.5	53.2	93.7
	50	5.9		14.6		18.6	
	40	2.0		5.7		6.1	
	25	—		1.2		1.0	

*Precipitate is formed after mixing.

Influence of the ionic strength

The influence of the ionic strength was examined in the range of $0.75 \times 10^{-2} - 1.625 \times 10^{-1}$ M with KCl[25]. Reaction rate measurements were carried out at a temperature range of 25-60 °C and at a pH range of 4.74-5.25. It was observed that the rate constant values decrease slowly as the salt concentration increases and that the differences are slightly reduced as the acceptor ability of the substituent increases. This infirms the former conclusion that a reaction of the polar form of the aroylhydrazine with the copper(II) ions takes place.

Substituent effect

In the chelate complex which is formed after mixing of the reactants[26], more favorable is the O - Cu(II) bond[27,28] and the electron is transferred by the π^* antibonding orbital of the C-O bond. Thus if any factor could affect the fractional

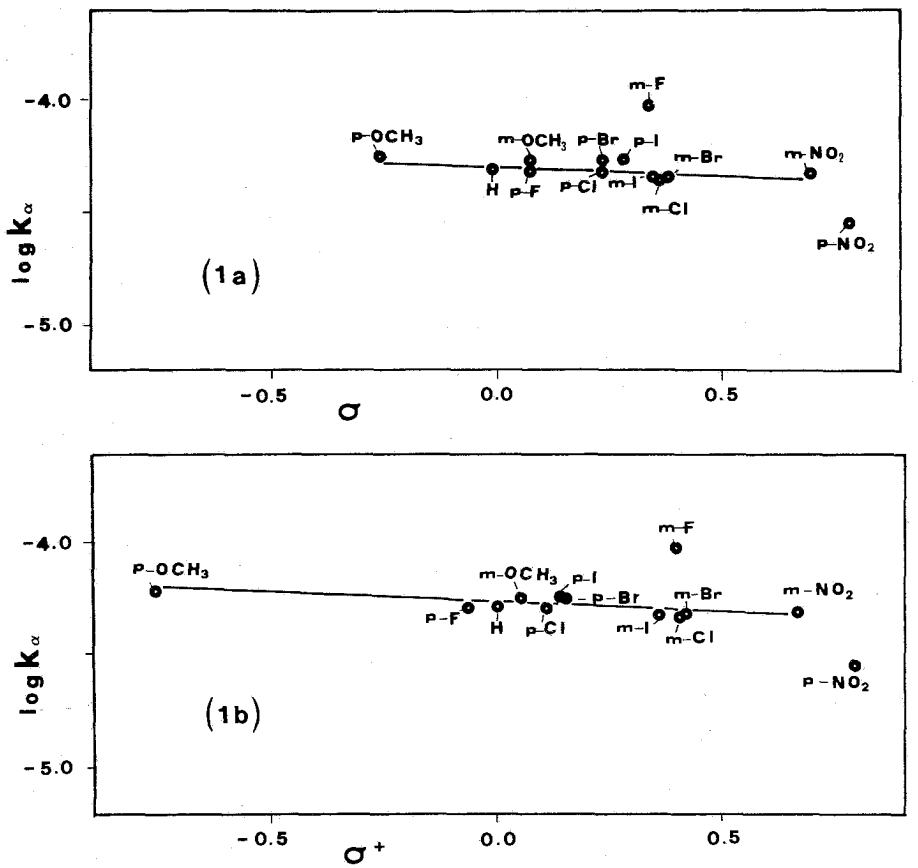

FIG. 1 : *The reaction rate constants k_α/s^{-1} of the oxidation of aroylhydrazines by copper(II) chloride as a function of the Hammett's parameters σ (Fig. 1a) and Brown's parameters σ^+ (Fig. 1b).*

charge of the oxygen, this factor could also affect, somehow, the reaction rate. From this point of view considerable factors could be the inductive and the resonance effects of the substituents.

As is obvious from Table I the k_α values of the p-XBH (except that of p-NO_2-BH) are almost the same whereas the differences among the corresponding E_α values are minimized. Both, k_α and E_α values of the p-XBH are considerably higher than those of the benzoylhydrazine. This suggests that the contribution of the resonance effect of the substituent at p-position is more significant than that of the inductive effect.

An attempt for the better evaluation of the influence of the inductive and the resonance effects of the substituents upon the reaction rate was afforted by means of the Hammett[29] and Brown[30] parameters correlated with the k_α values of the temperature of 40 °C (Fig. la and 1b). It is apparent from both diagrams (expect for the strongly deviated m-F-BH and p-NO_2-BH) that the dependence of the reaction rate on the substituent is small. Indeed, the value of ρ of the Hammett's equation is $\rho = -0.10$. Note, the correlation coefficient for the Brown parameters is higher than that of the Hammett's ($r = 0.924$ and $r = 0.816$ respectively). Thus the reaction seems to be favored by substituents which act as donors and which enhance the electron charge of the carbonyl oxygen[31].

In conclusion the substituents act rather upon the ability of the aroylhydrazine to coordinate with the Cu(II) ions whereas the contribution to the electron transfer rate is, possibly, negligible. The observed deviation of the m-F-BH can be attributed to transmolecular hydrogen bonds or even to a possible change of the reaction mechanism. The deviation of the p-NO_2-BH can be attributed to the resonance structure $^-O_2N =$  –C(O)$NHNH_2$ in which the donor ability of the carbonyl group has been strongly decreased[32].

Περίληψη

Κινητική μελέτη της οξείδωσης μονοϋποκατεστημένων στον πυρήνα αροϋλοϋδραζινών. Εφαρμογή της εξίσωσης Hammett.

Μελετήθηκε με τη μέθοδο της φασματοφωτομετρίας η κινητική της οξείδωσης δεκαοκτώ μονοϋποκατεστημένων στον πυρήνα αροϋλοϋδραζινών. Σαν οξειδωτικό χρησιμοποιήθηκε χλωριούχος χαλκός (II). Οι αροϋλοϋδραζίνες που χρησιμοποιήθηκαν είναι οι F, Cl, Br, I, NO_2 και OCH_3 μονοϋποκατεστημένες σε ο-, μ- και π- θέση. Η μελέτη έγινε σε θερμοκρασίες μεταξύ 25 και 60 °C και σε περιοχή pH 4.40 - 5.60 με τη χρησιμοποίηση οξικών ρυθμιστικών.

Η αντίδραση είναι πρώτης τάξης τόσο για τις αροϋλοϋδραζίνες όσο και για τον χλωριούχο χαλκό (II) και ακολουθεί το μηχανισμό μεταφοράς ενός ηλεκτρονίου. Η ταχύτητα της αντίδρασης ελαττώνεται γραμμικά με την αύξηση της συγκέντρωσης των ιόντων υδρογόνου. Οι τιμές της ενέργειας ενεργοποίησης

της αντίδρασης κυμαίνονται μεταξύ 79.5 και 110.0 kJ.mol^{-1} ενώ στις π- αροϋλοϋδραζίνες είναι παραπλήσιες κυμαινόμενες μεταξύ 91.6 και 96.2 kJ.mol^{-1}.

Η εφαρμογή της εξίσωσης Hammett έδειξε ότι η επίδραση του υποκαταστάτη στην ταχύτητα της αντίδρασης είναι μικρή ($\rho = -0.10$) η δε αντίδραση ευνοείται από υποκαταστάτες δότες ηλεκτρονίων.

Γενικά συμπεραίνεται ότι οι υποκαταστάτες επηρεάζουν κυρίως την ικανότητα συναρμογής των αροϋλοϋδραζινών με τα ιόντα χαλκού (II) ενώ η επίδρασή τους στο μηχανισμό μεταφοράς του ηλεκτρονίου είναι αμελητέα.

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ÜBER DIE INHIBIERENDE WIRKUNG VON TRIPHENYLARSINOXID AUF DIE POLAROGRAPHISCHE REDUKTION VON KATIONEN IN WASSER-METHANOLISCHEN UND METHANOLISCHEN LÖSUNGEN

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Zusammenfassung

Es wird über die inhibierende Wirkung von Triphenylarsinoxid (TAsO) auf die polarographische Reduktion von Cd^{2+}-, Zn^{2+}- und Co^{2+}-Ionen in wässerig-methanolischen und methanolischen Lösungen berichtet. In wässerig-methanolischen Lösungen war die Wirkung des Inhibitors stärker als in methanolischen Lösungen. Die grösste inhibierende Wirkung des Triphenylarsinoxids wurde bei der Reduktion von Zn^{2+}- und Co^{2+}-Ionen in H_2O/MeOH Lösungen bei einem Potentialbereich zwischen -1,0 und -1,3 V beobachtet. Die Wirkung von TAsO auf die Reduktion der Cd^{2+}-Ionen war vernachlässigbar klein.

Key Words : Polarography, Electroreduction, Inhibitors, Absorption Potential, Halfwave Potential, Triphenylarsine Oxide.

Abbreviations : TAsO: Triphenylarsinoxid, TPO: Triphenylphosphinoxid.

Einleitung

In früheren Arbeiten[1,2] hatten wir das eigenartige polarographische Verhalten von Triphenylarsinoxid (TAsO) festgestellt. Es konnte nämlich bewiesen werden, dass sich diese grenzflächenaktive Substanz, besonders im Vergleich mit anderen ähnlichen grenzflächenaktiven Stoffen, bei einem Potentialbereich von ungefähr -1,0 Volt in wässerig-methanolischen und methanolischen Lösungen sowohl als ein reiner Depolarisator, bei Anwesenheit von Protonendonatoren in der Lösung, als auch als ein Inhibitor von Elektrodenvorgängen verhält. Demzufolge, und wegen des öfteren Gebrauchs dieser Stoffe als Inhibitoren von Elektrodenprozessen,

wurde dann später in einer vergleichbaren Weise die Wirkung dieser Substanzen auf die polarographische Reduktion von Kationen untersucht[3].

Die Ergebnisse dieser früheren Arbeiten über TAsO[1,2] und die sehr wenigen Informationen, die in der Literatur über die inhibierende Wirkung dieser Substanz auf die Elektroreduktion von Kationen zu finden sind, veranlassten uns, das Verhalten dieses Inhibitors auf die polarographische Reduktion von Cd^{2+}-, Zn^{2+}- und Co^{2+}-Ionen an der Hg-Tropfelektrode in wässerig-methanolischen und methanolischen Lösungen eingehender zu untersuchen.

Experimentelles und Ergebnisse

Der experimentelle Teil dieser Untersuchung wurde in einer früheren Arbeit ausführlich beschrieben[3]. Alle Messungen wurden bei einer Temperatur von 25 °C durchgeführt.

Im Bereich der polarographischen Reduktion von Cd^{2+}-Ionen ($E_{1/2} = -595$ mV) in reinem Methanol zeigte das TAsO – sogar bei hohen Konzentrationen dieser grenzflächenaktiven Substanz ($5 \cdot 10^{-3}$ M) im Vergleich mit der entsprechenden Depolarisator-Konzentration in der Lösung (10^{-3} M) – keine inhibierende Wirkung. Verschiebungen dagegen der polarographischen Reduktionskurve des Depolarisators der Grössenordnung von 5 mV, in Lösungen mit 20 Vol-% H_2O und bei einer fünffachen Konzentration des Inhibitors ($5 \cdot 10^{-3}$ M) im Vergleich mit der entsprechenden Depolarisator-Konzentration reichen auch nicht aus, um eine inhibierende Wirkung von TAsO in wässerig-methanolischen Lösungen bei diesem Potentialbereich zu begründen. Diese Ergebnisse lassen sich sehr gut mit Hilfe der Resultate, erklären[4], die man aus Kapazitätsmessungen in ähnlichen Lösungssystemen erhalten konnte.

Relativ schwach erscheint die Wirkung dieser grenzflächenaktiven Substanz auf die polarographische Reduktion der Zn^{2+}-Ionen ($E_{1/2} = -1030$ mV) in reinem Methanol. Auch in diesem Fall kann die beobachtete Verschiebung der Reduktionswellen von 10 mV, bei einer fünffachen Konzentration des Inhibitors ($5 \cdot 10^{-3}$ M) im Vergleich mit der Depolarisator-Konzentration in der Lösung, keine nennenswerte inhibierende Wirkung begründen. Anhand der Kapazitätsmessungen und der Messungen des Bedeckungsgrades Θ sollte eigentlich in diesem Fall die Wirkung des Inhibitors etwas grösser sein, was man aber in reinem Methanol nicht feststellen konnte.

Eine stärkere Wirkung zeigt dagegen der Inhibitor auf die polarographische Reduktion desselben Depolarisators in wässerig-methanolischen Lösungen. Die Adsorptionsfähigkeit von TAsO auf der Elektrodenoberfläche steigt nämlich mit steigender H_2O-Konzentration in der Lösung, was man auch schon bei anderen Inhibitoren festgestellt hat[4-6], und demzufolge ist die Wirkung dieser grenzflächenaktiven Substanz in H_2O/MeOH-Lösungen grösser als in reinem Methanol. Eine solche Wirkung wäre eigentlich, anhand Hauptadsorptionsbereichs von TAsO und des Bedeckungsgrades Θ der Elektrodenoberfläche bei diesem Potentialbereich[4] zu erwarten.

Aus den entsprechenden Halbstufenpotentialverschiebungen $\Delta E_{1/2}$ wird deutlich, dass die Wirkung des Inhibitors mit steigender H_2O-Konzentration in der Lösung stärker wird. Diese Wirkung wird aber erst ab eine H_2O-Konzentration von 20 Vol-% deutlich. Bis zu dieser H_2O-Konzentration kann die Wirkung des Wassers auf die Halbstufenpotentialverschiebung $\Delta E_{1/2}$ als sehr klein und konstant angesehen werden. Bei noch höheren Wasser-Konzentrationen verlaufen die Halbstufenpotentialverschiebungen annähernd linear mit der H_2O-Konzentration in der Lösung, wie man auch aus Abb. 1 deutlich erkennen kann.
Eine ähnliche lineare Abhängigkeit konnte auch bei Anwesenheit von Triphenylphosphinoxid (TPO) in der Lösung beobachtet werden. Auch diese Abhängigkeit wird graphisch in Abb. 1 dargestellt.

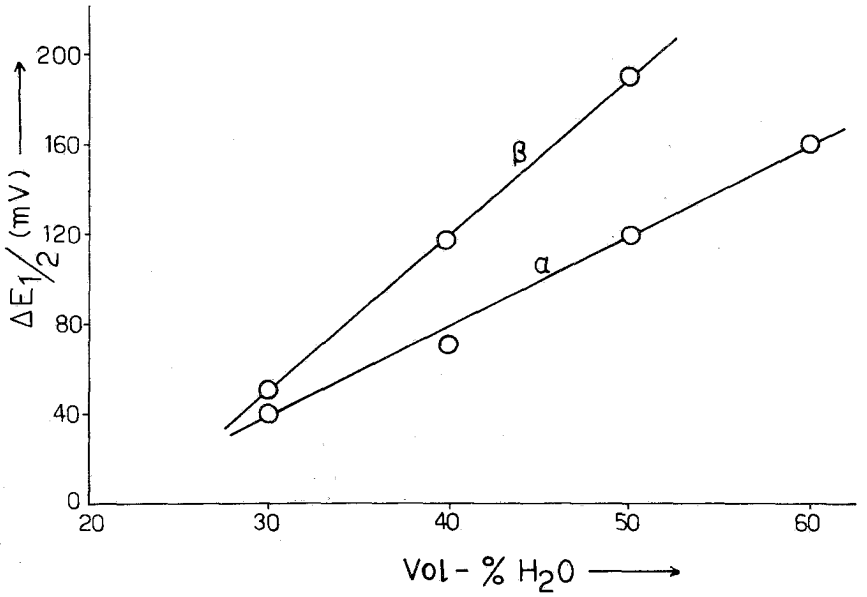

Abb. 1 : *Abhängigkeit der Halbstufenpotentialverschiebungen $\Delta E_{1/2}$, bei der Reduktion von Zn^{2+}-Ionen in wässerig-methanolischen Lösungen, von der H_2O-Konzentration in der Lösung und bei Anwesenheit von a) TAsO ($1 \cdot 10^{-3}$ M), b) TPO ($1 \cdot 10^{-3}$ M).*

Weitere Informationen, bezüglich der inhibierenden Wirkung von TAsO, konnten bei der polarographischen Reduktion von Zn^{2+}-Ionen in wässerig-methanolischen Lösungen und bei verschiedenen Inhibitor-Konzentrationen in der Lösung erhalten werden. In Abb. 2 werden als Beispiel die polarographischen Reduktionskurven des obengenannten Depolarisators in wässerig-methanolischen Lösungen mit 30 Vol-% H_2O bei verschiedenen Konzentrationen des Inhibitors wiedergegeben.
Wie es aus der obigen Abbildung deutlich wird, kommt die inhibierende Wirkung von TAsO in diesem Fall sowohl durch die Verschiebung der Kurven mit

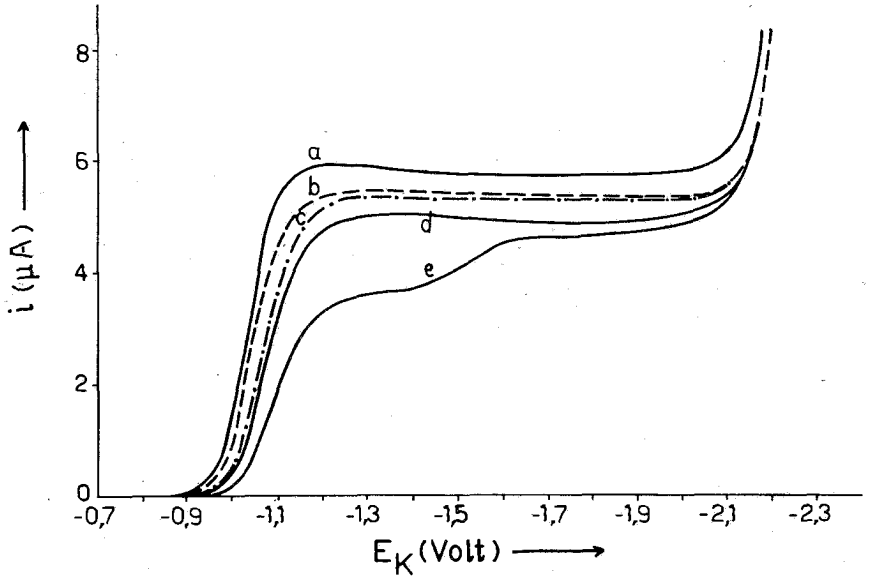

Abb. 2 : *Stromspannungskurven von $ZnSO_4 \cdot 7H_2O$ (10^{-3} M) in H_2O/MeOH-Lösungen mit 30% v/v H_2O a) ohne Inhibitor, b) 10^{-3} M, c) $2 \cdot 10^{-3}$ M, d) $4 \cdot 10^{-3}$ M, e) 10^{-2} M TAsO.*

wachsender Inhibitor-Konzentration in der Lösung, als auch durch die gleichzeitige Abnahme der Grenzstromstärke der Reduktionswellen zum Ausdruck. Bei grösseren Konzentrationen des Inhibitors beobachten wir infolge der Einwirkung der grenzflächenaktiven Substanz sogar eine Teilung der einfachen Stufe des Depolarisators in zwei Wellen (Abb. 2e). Schliesslich kann aus der obigen Abbildung, je mehr man zu grösseren Konzentrationen des Inhibitors übergeht, auch eine gleichzeitige Abnahme der Steigung der Reduktionswellen festgestellt werden, die auf die Zunahme der Irreversibilität des entsprechenden Elektrodenvorgangs deutet.

Es soll an diesem Punkt erwähnt werden, dass bei der Untersuchung derselben Systeme in Lösungen mit kleineren H_2O-Konzentrationen, wie z.B. bei 20 Vol-% H_2O, keine Abnahme der Grenzstromstärke, sogar bis zu einer vierfach grösseren Konzentration des Inhibitors ($4 \cdot 10^{-3}$ M) als diejenige des Depolarisators, bemerkt werden konnte. Auch in diesem Fall kann man also mit Sicherheit behaupten, dass in wässerig-methanolischen Lösungen die Wirkung von TAsO erst bei grösseren H_2O-Konzentrationen stark zum Ausdruck kommt.

Man könnte also annehmen, dass sich das TAsO bei diesem Potentialbereich erst bei einem grösseren Wassergehalt der Lösung als 20 Vol-% H_2O, wie erwartet, als ein relativ starker Inhibitor aufweist.

Im Bereich der polarographischen Reduktion der Co^{2+}-Ionen ($E_{1/2}$= ca.-1200 mV) zeigte das TAsO sowohl in methanolischen, als auch in wässerig-methanolischen Lösungen eine starke inhibierende Wirkung. Bei diesem Potentialbereich befindet sich nämlich der Inhibitor innerhalb seines Hauptadsorptions-

bereichs auf der Elektrode und der Bedeckungsgrad Θ der Elektrodenoberfläche ist gleichzeitig bei diesem Potentialbereich relativ gross[4]. Es wäre also eine starke Wirkung zu erwarten.

In Abb. 3 werden als Beispiel die polarographischen Kurven des Depolarisators in wässerig-methanolischen Lösungen mit 30 Vol-% H_2O und bei verschiedenen Inhibitor-Konzentration wiedergegeben.

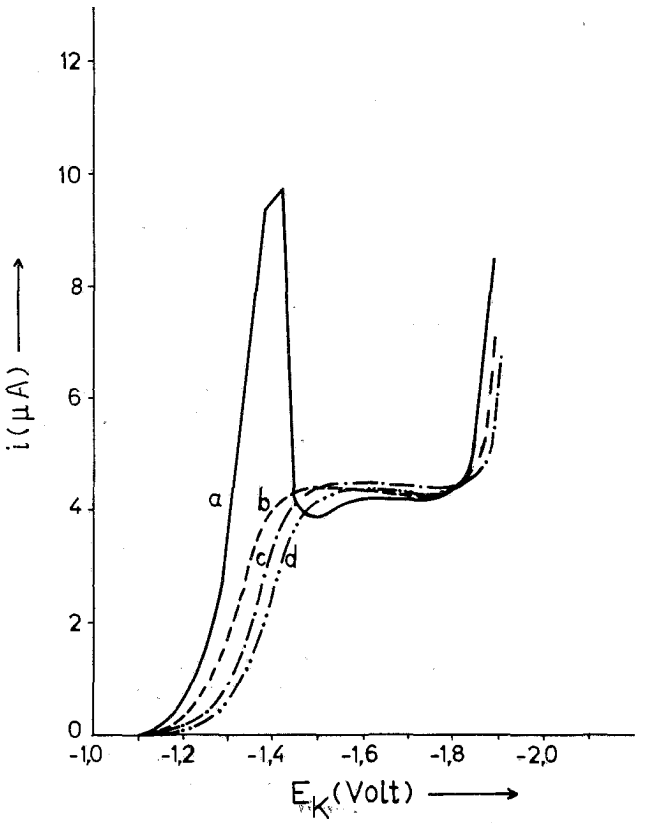

Abb. 3 : *Stromspannungskurven von $Co(NO_3)_2 \cdot 6H_2O$ ($0{,}9 \cdot 10^{-3}$ M) in H_2O/MeOH-Lösungen mit 30% v/v H_2O a) ohne Inhibitor, b) $2 \cdot 10^{-3}$ M, c) $4 \cdot 10^{-3}$ M, d) 10^{-2} M TAsO.*

Im Gegensatz zur Abb. 2 konnte in diesem Fall bei grösseren Konzentrationen des Inhibitors (Abb. 3d) keine Teilung oder Abnahme der Höhe der einfachen Stufe des Depolarisators beobachtet werden. Wie aber aus der obigen Abbildung durch die Verschiebung der Kurven deutlich zu erkennen ist, verhält sich das TAsO in diesem Fall – in H_2O/MeOH-Lösungen, bei diesem Potentialbereich und bei den angegebenen H_2O- und TAsO-Konzentrationen – als ein relativ starker Inhibitor des untersuchten Elektrodenprozesses.

Ein ähnliches Verhalten zeigt TAsO auf die polarographische Reduktion desselben Depolarisators in reinem Methanol. Auch in diesem Fall konnte anhand

der entsprechenden polarographischen Kurven eine starke inhibierende Wirkung beobachtet werden. Die Wirkung des Inhibitors scheint in diesem Fall grösser zu sein als im Fall der Reduktion der Zn^{2+}-Ionen im selben Lösungssystem.

Man kann also zusammenfassend annehmen, dass das TAsO, besonders in wässerig-methanolischen Lösungen mit hohen H_2O-Konzentrationen, bei einem Potentialbereich zwischen ca. -1,0 und -1,3 Volt eine relativ grosse inhibierende Wirkung auf die polarographische Reduktion der obengenannten zweiwertigen Kationen aufweist.

Summary

On the Inhibition Effect of Triphenylarsine Oxide on the Polarographic Reduction of Cations in Aqueous Methanolic and Methanolic Solutions

Data of the inhibition effect of triphenylarsine oxide (TAsO) on the polarographic reduction of Cd^{2+}, Zn^{2+} and Co^{2+} ions in aqueous-methanolic and methanolic solutions are reported.

The action of the inhibitor was always stronger in aqueous-methanolic than in methanolic solutions. The greatest inhibition effect of triphenylarsine oxide was obtained for the reduction of Zn^{2+} and Co^{2+} ions in H_2O/MeOH solutions at a potential range between -1,0 and -1,3 volts. The action of TAsO on the reduction of the Cd^{2+} ions was negligible.

Περίληψη

Μελέτη της παρεμποδιστικής δράσεως του Τριφαινυλοαρσινοξειδίου κατά την πολαρογραφική αναγωγή κατιόντων σε υδατομεθανολικά και μεθανολικά διαλύματα.

Μελετάται η παρεμποδιστική δράση του Τριφαινυλοαρσινοξειδίου (TAsO) κατά την πολαρογραφική αναγωγή των ιόντων Cd^{2+}, Zn^{2+} και Co^{2+} σε υδατομεθανολικά και μεθανολικά διαλύματα.

Η δράση του παρεμποδιστή ήταν ισχυρότερη σε υδατομεθανολικά διαλύματα από ό,τι σε διαλύματα με καθαρή μεθανόλη. Η μεγαλύτερη παρεμποδιστική δράση του Τριφαινυλοαρσινοξειδίου παρατηρήθηκε κατά την αναγωγή των ιόντων Zn^{2+} και Co^{2+} σε υδατομεθανολικά διαλύματα σε μια περιοχή δυναμικού μεταξύ -1,0 και -1,3 V. Η δράση του TAsO κατά την αναγωγή των ιόντων Cd^{2+} ήταν αμελητέα.

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Chimika Chronika, New Series, 12, 145-153 (1983)

THE REACTION COURSE AND KINETICS OF THE CATALYTIC WAVE OF THIOGLYCOLIC ACID (MERCAPTOACETIC ACID).

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Summary

The polarographic investigation of the catalytic hydrogen wave of thioglycolic acid presented in this work has enabled the interpretation of the course of the electrode reaction and the calculation of the velocity constant of the reaction. At the surface of the electrode a simultaneous reaction of the H^+ and $Ni(NH_3)_4^{2+}$ ions occurs, whereby the resulting interfacial tension effects are causing an increase of the current.

Key words: Thioglycolic Acid, Catalytic Wave Kinetics.

Introduction

Many organic compounds containing the -SH group are showing a «catalytical hydrogen wave» when polarographic measurements are carried out in buffered solutions containing amino-complexes of Co^{2+}, Co^{3+} and Ni^{2+}. The catalytic effect was discovered by Brdicka [1,2], and was subsequently applied for qualitative and quantitative measurements of organic compounds containing the -SH group. Similar effects with other organic compounds were also observed, and the ability to cause a catalytic hydrogen evolution was connected with the presence of unshared electron pairs at nitrogen, sulfur, phosphorus, arsenic and other atoms, to which a proton can be added [3].

From the works presented in the literature [4,5,6,7] it is possible to quote the following polarographic characteristics of thioglycolic acid. The thioglycolic acid can react at the Hg-electrode by receiving or giving electrons, and accordingly the measurements might show three kinds of polarograms :

a) the anodic oxidation wave
b) the cathodic reduction wave
c) the catalytic reduction wave

a)According to literature data, the anodic wave is caused by a reaction of Hg with the -SH group :

$$RSH + Hg \rightarrow RSHg + H^+ + e$$

b) The cathodic wave is a result of the hydrogen ion reduction from thioglycolic acid i.e. from its carboxylic group and the -SH group, depending upon the pH of the solution[4,5,6,7].

c) The catalytic hydrogen wave of thioglycolic acid results as an increased reduction wave, when measurements are performed with solutions containing amino-complexes of Co^{2+}, Co^{3+} or Ni^{2+} (Brdicka solutions). Based upon many experimental investigations (Literature review in 3.) a theory of the catalytical hydrogen evolution was developed. The theory proposed by Mairanovskij [3]assumes the same reaction course of the catalytic hydrogen wave for sulfur containing compounds as for nitrogen containing organic bases, i.e. a reaction in 3 steps :

- A protonation of the catalyst
- The electron transfer upon the protonated catalyst
- The release of a molecule of hydrogen from two reduced protonated «onium» cations, in a second order reaction.

However, according to the statement of Mairanovskij [3], the available experimental evidence was not sufficient to draw final conclusions about the reduction process of hydrogen, catalysed by compounds containing the -SH group.

Because several aspects of the mechanism of this process have not yet been clarified, numerous investigators continued to investigate the reaction course of the catalytic waves of mercapto-compounds[8,9,10,11,12]. In their papers the investigators assume that catalytically active centers are formed by adsorption of organic catalysts on deposited Co, or the formation of complexes with Co(II) or Ni(II).

In this work a new theory of catalytic hydrogen waves is presented based upon a previously established mechanism of hydrogen evolution[13]. The experimental measurements in favor of the theory are D.C. polarograms recorded in systems containing thioglycolic acid and LiCl, with or without Ni-amine complexes (Brdicka solutions), and electrocapillary curves recorded by a modified technique in this solutions.

Experimental

The polarograms were recorded by a polarograph PO 4 «Radiometer» Kopenhagen. Because reactions proceeding at the surface of the Hg-electrode were investigated, the purification of mercury was performed with special care. It was cleansed chemically by boiling small portions of Hg with 20% NaOH, washing with water, whereupon the Hg was dispersed through a 2 m column containing hot 10% HNO_3, collected again and washed thoroughly with water. The principal purification was the distillation. The triply distilled mercury had a light colour and did not form ring sediments on glass containers.

The Hg-electrode had the following characteristics: $m = 1{,}39\ mg.s^{-1}$, $t = 5{,}37$ s, in 10^{-1} M KCl, at a potential of 0 Volts vs. S.C.E.

The water used for the preparation of solutions was triply distilled. All chemicals were of analytical grade pyrity. The measurements were performed at constant temperature of 25°C (± 0,2°) by immersion of the polarographic cell and the reference electrode (S.C.E.) in a thermostat.

Results

For the polarographic measurements LiCl was used as supporting electrolyte, because the Li^+ ion, having a very negative reduction potential (-2,9 V vs. S.C.E.) enabled the observation of polarographic waves in a greater potential span.

In thioglycolic acid solutions containing LiCl as supporting electrolyte, two waves are formed, the first wave of thioglycolic acid at -1,6 V and the second at -2,2 V.

The first figure 1 is showing both waves in comparison with the residual current recorded in a solution of pure LiCl of the same concentration. It will be noted that the residual current does not show any wave just preceding the rapid increase of the Li^+ reduction current.

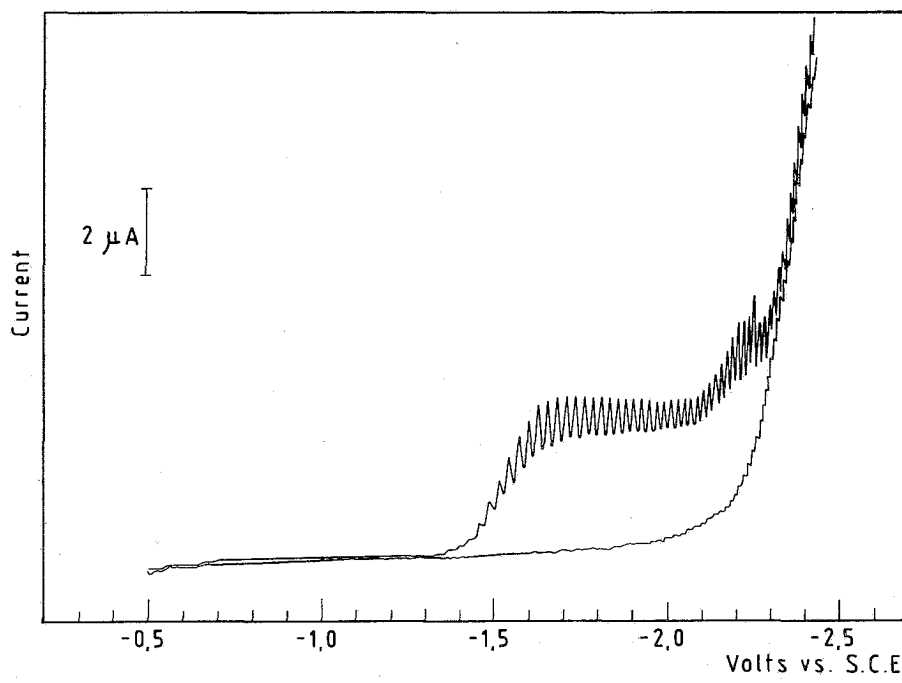

FIG. 1 : *A polarographic wave of* 4.10^{-4} $SHCH_2COOH$ *in* 10^{-2} *M LiCl, in comparison with the supporting electrolyte.*

Numerous scientific works are mentioning the first wave, whereas the second wave was unnoticed. The second wave has a noncompletely developed form

because of the overlapping with the Li^+ reduction wave. Therefore, the height of such a wave does not depend solely upon the concentration of $SHCH_2COOH$, but upon the ratio of the $SHCH_2COOH$ and LiCl concentrations, as well as the first completely developed wave.

By the addition of Ni(II) amino complexes in the solution, the first wave is increased, i.e. a «catalytic hydrogen wave» is formed (Figure 2).

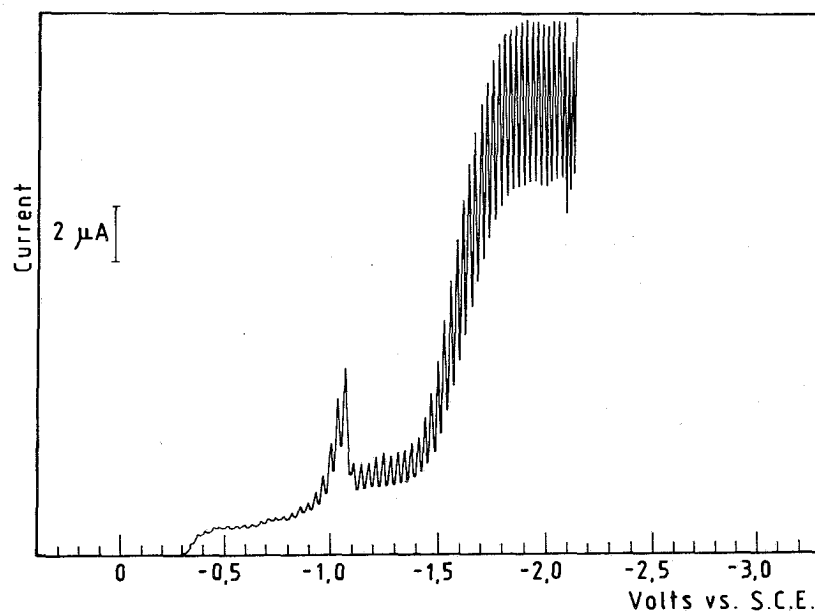

FIG. 2 : *A polarographic wave of* 2.10^{-4} *M* $SHCH_2COOH + 10^{-2}$ *M LiCl* $+ 10^{-4}$ *M* Ni^{2+} $+$ *buffer* 10^{-1} *M* $NH_4Cl + 10^{-1}$ *M* NH_3.

The wave preceding the catalytic wave is a reduction wave of Ni(II) with a maximum caused by a diproportionation reaction.

In the solution corresponding to the first and second figure, interfacial tension measurements were also carried out. The usual technique of drop – time measurements of the Hg-electrode was modified, inasmuch as two capillaries of different diameters have been used for measurements, connected in λ-shape. The difference of the drop-time of the two capillaries vs. potential is shown on Figure 3., in comparison with the electrocapillary curve recorded by the same technique in the solution of the supporting electrolyte LiCl.

The advantage in measuring the difference of the drop time is based upon the fact, that the force causing the surface tension can be resolved in two components, the perpendicular and the tangential component, when reaction steps are occurring tangential with the electrode surface. (Figure 4).

When measuring the surface tension conventionally, the effects upon the perpendicular forces are predominant, i.e. the forces arising from the charging of

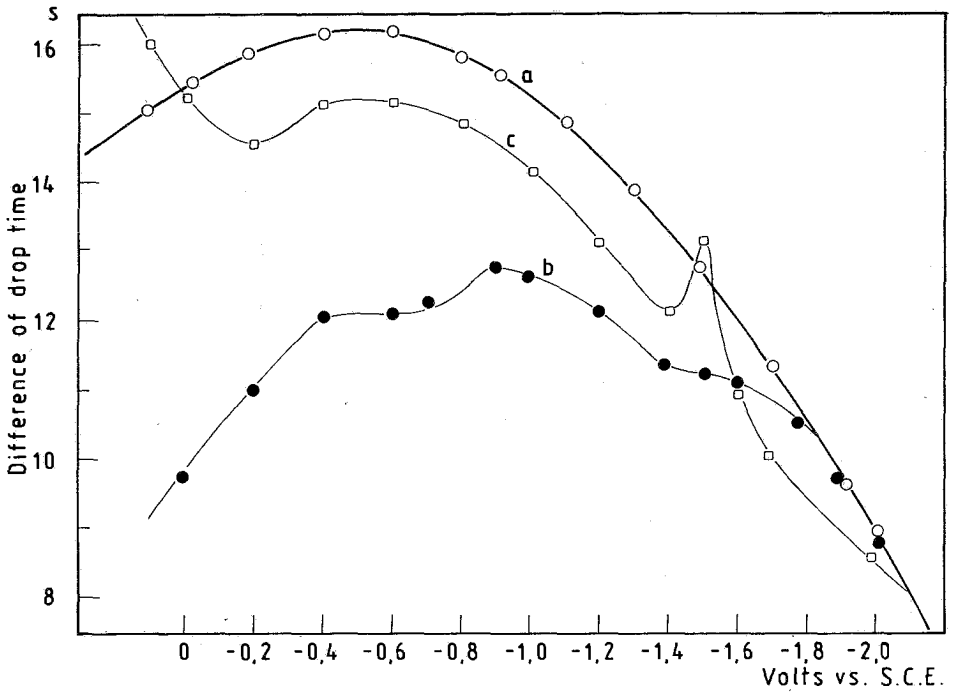

FIG. 3 : *Interfacial tension measurements corresponding the polarographic waves shown on Figures 1. and 2.*
a) 10^{-2} *M LiCl*
b) 4.10^{-4} *M* $SHCH_2COOH + 10^{-2}$*M LiCl*
c) 2.10^{-4} *M* $SHCH_2COOH + 10^{-2}$*M LiCl* $+10^{-4}$ *M* Ni^{2+} *+ buffer* 10^{-1} *M* $NH_3 +$ $+ 10^{-1}M\,NH_4Cl$

the electric double layer. In contrast, when measuring the difference of the drop time, the effects upon the perpendicular component are minimized, because both electrodes have the same potential, and the effects of the tangentially transferred charges are revealed.

Therefore maxima appear upon the electrocapillary curves at potentials corresponding to catalytical waves, to maxima of the first kind, or similar processes involving a rearrangement of charged particles upon the electrode surface.

Heyrovsky and Kučera have shown a similar modification of the electrocapillary curves by measuring the drop weight[4] («Principles of Polarography» p. 23). They have also observed secondary maxima upon such electrocapillary curves.

The measurements of the tangential surface tension shown in this work, bring to light an increase of the surface tension at potentials of the catalytic wave, when $Ni(NH_3)_4^{2+}$ is present in the solution, whereas in the absence of $Ni(NH_3)_4^{2+}$, a tendency of decreasing surface tension is observed.

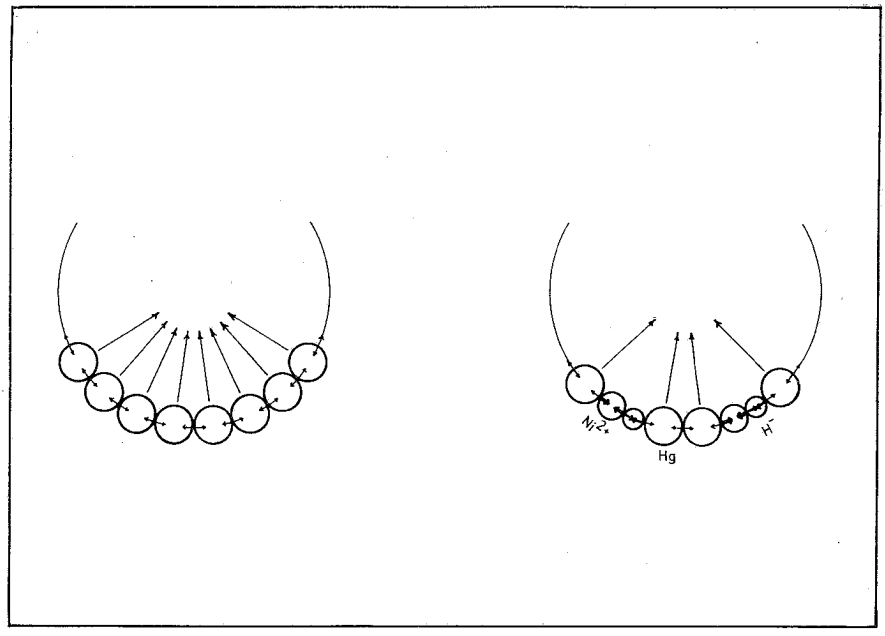

FIG. 4 : *Arrangement of particles at the surface of the mercury electrode :*
a) solutions in the absence of Ni^{2+}
b) solutions in the presence of Ni^{2+}

The reaction course

The two waves of thioglycolic acid described in this work, are interpreted by a reaction course generally accepted for the electrode reaction of H^+ ions [13].

In the first step, the thioglycolic acid is receiving two electrons from the electrode :

$$SHCH_2COOH + 2e \rightarrow SHCH_2COO^- + H^- \qquad (1)$$

thereby forming hydride ions, stabilized by the formation of LiH. The hydride ion is in turn acting spontaneously with H^+ ions; resulting in the evolution of hydrogen :

$$H^+ + H^- \rightarrow H_2 \qquad (2)$$

Reaction (2) is proceding in the aqueous layer, adhering the surface of the electrode, and it is accompanied by a release of energy. The energy released enables the formation of a prewave. Therefore, the reduction wave observed at -1,6 V is a prewave, and the second uncompletely developed wave at -2,2 V is the wave of the hydride ion.

The catalyzed evolution of hydrogen discernable as the increased wave at -1,6V, is formed by the intermediately deposited Ni° :

$$Ni(NH_3)_4^{2+} + 2e \rightarrow Ni^{\circ} + 4NH_3 \quad (3)$$

The elementary Ni° is further effective by a reaction involving two electrons :

$$Ni^{\circ} + H^{+} \rightarrow Ni^{2+} + H^{-} \quad (4)$$

The alternative arrangement of particles with opposite charges, Ni^{2+} and H^{-}, at the surface of the electrode, causes the increase of the Hg drop, which is therefore able of conducting a greater current.

The above described reaction course offers interpretation for the formation of the hydrogen wave at more positive potentials, i.e. the energy component of the catalytic effect, whereas the increase of the current is a consequence of the secondary effect upon the substrate (Hg-electrode). The effect upon the surface tension of the substrate is shown on Figure 3. curve c). in the form of the small maximum at the potential of -1,6 V.

Kinetics

The kinetic law in accordance with the described reaction course is based on a reaction of the second order :

$$H^{+} + H^{-} \rightarrow H_2$$

The velocity of the whole electrode process is controlled by the formation of molecular hydrogen from the hydrogen and the hydride ion.

The velocity constant was calculated by an expression derived in a previous work [14] :

$$I = n.F.k_s.C.10^{-\frac{nF}{2,3RT.2}(E - E_s + 0,0592 \log c_{Li^+})}$$

where the symbols have the following significance :

I = current density

n = the number of electrons tranferred effectively at the electrode i.e. 1 electron $\times$ 2/3 surface = 0,4 electrons

C = the $SHCH_2COOH$ concentration of the solution

k_s = the velocity constant corresponding to the half-wave potential of the thioglycolic acid wave.

E_s = the half-wave potential of the thioglycolic acid reduction

E = the half-wave potential of the small uncompletely developed wave.

c_{Li^+} = concentration of the supporting electrolyte, LiCl, effective for the activation energy.

The velocity constant for the first wave of thioglycolic acid is 5.10^{-6} cm.s^{-1}, as calculated by the above expression. This value is comparable with literature data [15].

Conclusion

The interpretation of the reaction course of the «catalytic hydrogen wave» i.e. the «Brdicka reaction» presented in this work, might offer aid in different applications of such reactions, e.g. for diagnostic purposes, therapeutic or preparative purpose. The velocity constant of the reduction reaction is smaller than the velocity constant of HCl by two orders of value, in accordance with the degree of dissociation of the acid.

The described technique of the surface tension measurement, represents an excellent tool in studying reactions proceeding stepwise, tangentially to electrode surfaces. The condition for reproducible results is a specially clean surface of mercury. The diagramms of the difference of drop time vs. potential are similar with the conventional drop time vs. potential diagramms [8], but are displaying additional effects of the tangentially proceeding electrode reaction steps.

Περίληψη

Η πορεία αντίδρασης και η κινητική του καταλυτικού κύματος του θειογλυκολικού οξέος.

Η πολαρογραφική έρευνα του καταλυτικού κύματος υδρογόνου του θειογλυκολικού οξέος που παρουσιάζεται σε αυτή την εργασία καθιστά ικανή την ερμηνεία της πορείας της αντίδρασης ηλεκτροδίου καθώς και τον υπολογισμό της σταθεράς ταχύτητας αντίδρασης. Στην επιφάνεια του ηλεκτροδίου -Hg λαμβάνει χώρα μια αυθόρμητη αντίδραση των ιόντων H^+ και $Ni(NH_3)_4^{2+}$, όπου τα αποτελέσματα της επίδρασης της διεπιφανειακής τάσης προκαλούν αύξηση του ρεύματος.

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SHORT PAPER

Chimika Chronika, New Series, 12, 155-160 (1983)

SYNTHESIS AND USE OF NEW, FIBRE-REACTIVE DYES SUITABLE FOR TRANSFER-PRINTING NYLON

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(Received June 28, 1982)

Introduction

Sublimation Transfer Printing, is a process where, selected sublimable dyestuffs, originally printed on paper, are transferred to suitable fabric held in contact with the paper, and raised to temperature from 150° to 220°C. It is possible to obtain the contact and heat required, with a hot-iron or a hot-press. Transfer times of 30 to 60 seconds are normally required, and excellent quality prints are usually obtained.

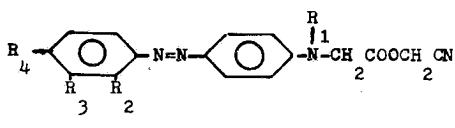
Since 1968, when this Sublimation Transfer Printing was commercially developed, there has been a dramatic and almost universal interest in this process, because of its simplicity and certain other advantages over the conventional textile printing.

Hitherto, the most successful application of this process, has been on polyesters and acetates. Polyamides give intense, bright prints, but the wash fastness properties of the print is in general unsatisfactory. To improve this various reactive dyes have been introduced, which give dye-fibre grafting during the transfer process, and give prints with very high wash fastness. Dyes bearing azide groups [1], epoxides [2], chloralkyl [3], α,β-unsaturated acyl [3], have been used, but the problem has been partially only solved. Difficulties have been encountered with the production of reds and blues, with adequate transferability [4], and consequently new suggestion are of significant importance.

The present study describes the synthesis of some new sublimable disperse dyes, bearing the cyanomethylester group as reactive group, and the potentiality of using them as fiber-reactive dyes, for transfer-printing nylon.

Experimental Part

Dyes belonging to two main classes were synthesized. Azo-dyes of the general formula I, and anthraquinone dyes of the general formula II. By varying the R_2, R_3 and R_4 substituents of the azo-dye, one can produce yellow, orange or red shades, while anthraquinone dyes of the formula II are blues.



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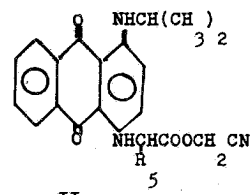


II

Preparation of the coupling components of the formula III. A modification of BASF process[5] *was used for this purpose.*

Two Moles of monoalkyl aniline, and one Mole of monochloroacetic acid, were heated in an oil bath, for three hours at 100 °C and then four hours at 115 °C. The reaction mixture was taken in water and the pH was adjusted to 10 with dilute sodium hydroxide solution. The unreacted monoalkylaniline was extracted with ether, the pH of alkylaniline acetic acid solution was adjusted to 7 with dilute HCl solution, and titrated in a small sample with N/1 diazotided p-nitroaniline solution. The coupling component solution was then used directly for azo-dye synthesis. The yield was almost quantitative.

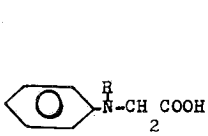
Synthesis of acid azo-dyes of the general formula IV.

A mixture of one Mole of the appropriate diazonium component, 100 ml water, and 30 ml 30% HCl solution, was cooled to 0 °C. A 30% solution containing one Mole of sodium nitrite was then added, and the reaction mixture was kept at 0-5 °C for one hour. After diazotization was completed, any excess of sodium nitrite was destroyed by the addition of small amount of sulfamic acid. The diazonium solution was then clarified and slowly added to a precooled to 5 °C solution, containing 1,05 Moles of coupling component. The reaction mixture was then stirred at 5-10 °C for three hours, the precipitated acid dye was redissolved by addition of 10% sodium hydroxide solution, and clarified from Celite. By careful acidification with dilute HCl, the dye was reprecipitated, filtered, washed with water and dried. Yields up to 95% were obtained.

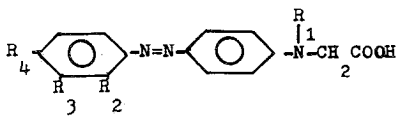
Synthesis of anthraquinone acid dyes of the formula V.

To a mixture of 0,0375 Moles quinizarin and 0,0135 Moles leucoquinizarin, water was added to a volume of 75 ml. Then there were added, 36 g isopropanol and the mixture was heated to 40 °C, with stirring. After addition of 0,055 Mole isopropylamine, 0,055 Mole glycine or alanine, and 0,055 Moles sodium hydroxide, the reaction mixture was brought slowly to boiling, and kept refluxing for eight

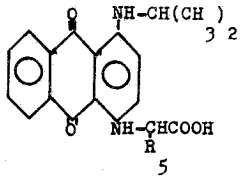
hours. 1,25 g sodium perborate was then added and the mixture was kept at boil for another two hours. The hot mixture was poured into 500 ml water, made alkaline with sodium carbonate, and the insoluble material was filtered off. The clear filtrate was then carefully acidified to pH 2 with dilute HCl solating, and the precipitated acid dye was filtered off, washed with water, and dried. The yield was 25%.



III



IV



V

General Procedure for the preparation of cyanomethyl active esters.

A mixture of 0,2 Mole of acid dye, 60 ml dry ethyl acetate, 0,24 Mole triethylamine, and 0,35 Mole chloroacetonitrile, was refluxed for ten hours. After evaporation of the solvent, the solid residue thus obtained, was washed with water, then stirred in a 10% sodium carbonate solution, and filtered off. This was repeated till the sodium carbonate washing remained colourless, and TLC chromatography showed no starting material. The cyanomethylester was then filtered, washed with water and dried in vacuum. Yields up to 75% were usually obtained. Samples for analysis were prepared by recrystallization from small amount of ethyl acetate.

The structure of the new dyes were very easily veryfied by NMR spectroscopy, since the α to carbonyl group protons, as well as the protons of cyanomethyl group, have characteristic absorption bands.

The following dye-active esters were synthesize:

1. Azo-dyes of formula I
   a) $R_1 = CH_2CH_2CN$, $R_2 = H$, $R_3 = R_4 = Cl$, hue yellow.
      calc. C = 54,82% H = 3,63% N = 16,82%
      found C = 54,53% H = 4,01% N = 17,02%
   b) $R_1 = CH_3$, $R_2 = R_3 = H$, $R_4 = NO_2$, hue orange.
      calc. C = 57,13% H = 5,35% N = 19,59%
      found C = 56,98% H = 5,12% N = 19,39%
   c) $R_1 = CH_3$, $R_2 = Cl$, $R_3 = H$, $P_4 = NO_2$, hue red.
      calc. C = 52,65% H = 3,63% N = 18,05%
      found C = 52,43% H = 3,97% N = 18,07%
2. Anthraquinone dyes of formula II.
   a) $R_5 = H$, hue blue.
      calc. C = 66,83% H = 5,07% N = 11,13%
      found C = 66,48% H = 5,39% N = 11,41%

b) $R_5 = CH_3$, hue blue

calc. C = 67,50% H = 5,40% N = 10,73%

found C = 67,34% H = 5,03% N = 10,49%

Evidence of reaction with nylon.

Two kinds of evidence were used to prove reaction of the dyes with nylon [6] :

1) Nylon dyed with conventional disperse, or acid dyes, can be stripped by boiling solvents such as, propanol, chlorobenzene, or aqueous 15% pyridine. On the contrary, nylon dyed with fibrereactive dyes, can not be stripped.
2) If nylon fibre, dyed with disperse dyes, is dissolved in 70% formic acid, and the resulting solution poured into propanol, the polymer is precipitated off, and the dye appears in the liquid phase. If, however, nylon dyed with fibre-reactive dyes is treated the same way, the dye remains associated with the precipitated nylon, even though it is readily soluble in propanol.

Transfer printing.

Nylon 6.6 was used as substrate to transfer printing.

The dyes were dissolved in acetone to give a saturated solution, and the solution was applied to filter paper. The filter paper was dried in the air and used as printing paper [1].

Each printing paper was placed in contact with the nylon substrate, and pressed with a hot iron at 200°C, for 45 seconds. The hot iron was in contact with the printing paper, and not with nylon.

After removing the filter paper, the substrate was left coloured with the dye used, in bright shades. The coloured substrate was then cut in equal pieces, and each piece was subjected to dry heat fixing by a hot at 180°C iron, the fixing time ranging from piece to piece from zero to two minutes.

When the fabric-samples were tested for dye-nylon reaction, by the tests described above, both tests showed the followings :

Almost no reaction for samples without dry-heat fixing, and gradual increase of chemically bunded dye, with increasing fixing times.

At two-minutes fixing time, only small amount of the dyestuff could be stripped from nylon.

When test No. 2 was applied, almost all color followed the precipitated polymer.

When, instead of nylon, polyester or polyacrylic substrates were used, the bright colored samples were completely stripped in boiling aqueous pyridine, even after dry-heat treatment of the samples for several minutes.

Kind of chemical bond.

The only functional group in polyamides that can react with an active ester, is the amine end-groups, and consequently the dye-fibre grafting can be understood according to the following scheme :

$$D\text{-}\underset{\displaystyle R}{\underset{|}{N}}\text{-}CH_2COOCH_2CN + NH_2\text{-nylon} \rightarrow D\text{-}\underset{\displaystyle R}{\underset{|}{N}}\text{-}CH_2CO\text{-}NH\text{-nylon} + HOCH_2CN$$

where D stands for the colored molecule.

The extend of dye-fibre grafting, reaches a maximum value, as a consequence of the limited end-groups of nylon. Additional dye is not chemically bonded, and therefore can be easily extracted with boiling propanol, or aqueous 15% pyridine.

Conclusion

Special, cyanomethylester bearing, disperse dyes, have a considerable potential for use as reactive, transfer-printing dyes, in particular for nylon substrates. They can also be used as regular disperse dyes, for any hydrophobic fibre, as polyester, acetates, acrylics, or blends. For the determination of the optimum condition for dye-transfer, as well as fixing, additional research work is in progress.

Abstracts

The preparation of some new reactive disperse dyes, which can be used for transfer-printing, is described. The dyes, azo and anthraquinone, bear the cyanomethylester group, which can react with amino-end groups of polyamides, and give non-extractable, dry-heat-transfered prints.

On hydrophobic fibres without functional groups, they behave like conventional disperse dyes.

Key Words : Disperse dyes, cyanomethyl ester, sublimation, amino-end group.

Περίληψη

Νέα, αντιδρώντα με την ίνα, χρώματα, χρήσιμα στην τυποβαφική του Νάϋλον με εξάχνωση.

Περιγράφεται η σύνθεση και χρήση νέων χρωμάτων διασποράς, τα οποία φέρουν στο μόριό τους τον ενεργό κυανομεθυλεστέρα.

Τα χρώματα αυτά ανήκουν σε δυο κατηγορίες. Αζωχρώματα του τύπου I και ανθρακινόνης του τύπου II. Με κατάλληλη επιλογή των υποκαταστατών των χρωμάτων του τύπου I, είναι δυνατόν να παραχθούν κίτρινες, πορτοκαλλί και κόκκινες αποχρώσεις. Τα χρώματα του τύπου II είναι μπλε.

Με εξάχνωση, τα νέα χρώματα, μπορούν να μεταφερθούν από διηθητικό

χαρτί στο οποίο έχουν προσροφηθεί, σε υφάσματα από υδρόφοβες ίνες. Τούτο επιτυγχάνεται όταν, το χαρτί, σε επαφή με το ύφασμα-υπόστρωμα, θερμανθεί στους 200°C επί 45-60 δευτερόλεπτα. Όταν το ύφασμα-υπόστρωμα είναι από Νάϋλον και μετά την εκτύπωση ακολουθήσει ξηρή θέρμανση στους 180°C, επιτυγχάνονται εκτυπώσεις σταθερές στο πλύσιμο και στους θερμούς οργανικούς διαλύτες. Δεν συμβαίνει το ίδιο με τα κοινά χρώματα διασποράς τα οποία εύκολα εκχυλίζονται.

Η σταθερότητα αυτή αποδίδεται στο σχηματισμό αμιδικού δεσμού του χρώματος με τις ελεύθερες αμινομάδες του πολυαμιδίου.

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SHORT PAPER

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OCCURRENCE OF PROSTAGLANDINS IN TETRAHYMENA PYRIFORMIS

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The possible presence of prostaglandins (PG) in *Tetrahymena pyriformis* as a means to counteract the effects of catecholamines has been long ago in question[1]. On the other hand, the presence in Tetrahymena of polyunsaturated fatty acids of the C-20 series, that is a prerequisite for PG biosynthesis, has been demonstrated since 1963[2]. Although these fatty acids were not unequivocally characterized, we were prompted to undertake the task to investigate whether prostaglandins do really occur in Tetrahymena. Convincing positive evidence was first obtained by gas-liquid chromatography (GLC). However, owing to the minute amounts of PG detected by GLC, these finding were cross-checked by radioimmunoassay (RIA) methods which, apart from their absolute specificity, are advantageous for the quantitation of minute amounts of prostaglandins.

Methods

Cultures (100 ml) of *T. pyriformis*, strain W, were prepared as previously described[3] in 2% proteose-pentone (Difco), 0.5% dextrose, 0.2% yeast extract (Difco) and 1% (v/v) of 90 μM ferrum-EDTA. At the age of 72 h after innoculation (cell density: 0.8×10^6 cells/ml), the cultures were cooled quickly to 4 °C in a dry ice-acetone bath, and the cells harvested by centrifugation at 750 xg (5 min) in a refrigerated centrifuge. The cells were washed free of medium by resuspension in ice-cold 0.9% NaCl followed by centrifugation as above. They were then suspended in 3 ml of 0.9% NaCl and extracted with 10 volumes of ethanol:ether, 3:1 (v/v)[4].

The culture media remaining after spunning down the cells, were centrifuged at 3,000 xg (10 min) to remove any residual cells and then, 10-ml aliquots were extracted as above.

The ether extracts were evaporated to dryness in vacuo and the dry residues were distributed in carbon tetrachloride: water, 1:1 (v/v). The aqueous layers were acidified to pH 3 with 0.1N HCl and extracted with ethyl acetate. The extracts were neutralized with 1 N NH_4OH, evaporated to dryness in vacuo and redissolved in 0.5 ml of benzene:ethylacetate, 60:40 (v/v). The resulting solutions were then fractionated on a 0.6 × 3 cm silicic acid chromatographic column[5], eluted with benzene:ethyl acetate:methanol 60:40:0 (5 ml), 60:40:2 (12 ml) and 60:40:20 (5 ml). The first solvent mixture eluted the PGA, PGB and undesirable lipids. PGE and PGF were then eluted with the second and third solvent mixture respectively. The performance of the column was tested prior to each series of experiments with radioactive (^{14}C) prostaglandins (Amersham, 160 Ci/mmole).

Derivatives for GLC analysis were prepared according to Albro and Fishbein[6] as follows: PGE methoximes were prepared by treatment of the PGE fractions with 0.3 ml of methoxamine hydrochloride in pyridine for 2h. The resulting methoximes, as well as the PGF fractions were treated with diazomethane in methanol:ether, 1:9 (v/v). The methylesters so obtained were converted to the corresponding trimethylsilyl derivatives by treatment for 20 min with 0.1 ml of a mixture of trimethylsilylimidazole (TSIM) (2 parts) and bis (trimethylsilyl) trifluoroacetamide (BSTFA) (1 part). GLC analysis was then performed on an OV.1 column (6ft × 1/8'' i.d.) using an Aerograph 200 apparatus with flame ionization detector.

Radioimmunoassays were carried out as described by Dray *et al.*[7] using antisera (M-anti-PGE_1, M-anti-PGE_2, L-antiPGF_{2a}) provided by the Institute Pasteur (Paris, France). Radioactive (carbon-14) prostaglandins (160 Ci/mmole) were purchased from Amersham. Nonradioactive prostaglandins were an offer from the Upjohn Company (Kalamazoo, Michigan).

TABLE I : Prostaglandin Levels in Cells, Growth Media and Cell Homogenates of Tetrahymena*

	PGF_{2a}	PGE_2	PGE_1
Cell mass	380 ± 160	270 ± 60	210 ± 40
Growth medium	2,100 ± 300	4,000 ± 500	3,300 ± 200
Cell homogenate:			
0 min	270	410	—
1 min	3,200	480	—
3 min	880	195	—
5 min	445	170	—
12 min	460	170	—
50 min	325	150	—

* Results are expressed in pg per 8×10^7 cells, or per 100 ml growth medium. *In situ* levels are average values ± SD from 11 assays. Results for cell homogenate are from one representative experiment.

Interestingly, the growth media of Tetrahymena cultures were found to contain PGE_2 and PGF_{2a} in concentrations much higher than in the cells, whereas sterilized plain medium gave negative results. These prostaglandins are slowly excreted from cells to medium during their growth, since negligible amounts of PGs were found in the media of cells collected by centrifugation and resuspended in fresh growth medium or in 0.9% KCl.

As shown in Table I, when cell homogenates are incubated at 37 °C, show a sudden increase of the PG levels. This is especially pronounced in the case of PGF_{2a} showing an almost 12-fold increase within one minute, followed by gradual decrease to normal levels within 3-4 min. A specific quick stimulation of the PGF_{2a} biosynthesis is a possible explanation for the observed difference between PGE_2 and PGF_{2a}. However, since PGF_{2a} is a metabolite of PGE_2, most probably the observed accumulation of PGF_{2a} is the result of both, reduction of PGE_2 and increased endoperoxide activity and cleavage.

Results and Discussion

The identification of PGs in Tetrahymena by GLC method has created serious problems due to artefacts formed during derivatization, in concentrations similar or greater than those of the PG-derivatives. Among several methods tried, it was shown that the preparation of the trimethylsilyl derivatives by treatment with TSIM-BSTFA [6] was advantageous in avoiding all the above problems. Using this technique it was shown unequivocally that prostaglandins E and F are present in Tetrahymena.

However, since GLC analysis gives no information about the components of the PGE and PGF fractions, this investigation was continued by RIA. As shown in Table I, Tetrahymena cells contain PGF_{2a}, PGE_2 and PGE_1 (in order of decreasing concentration).

The usual problems in estimating the *in situ* levels of PGs are more pronounced in this case, because in Tetrahymena cells extensive lipolysis (and apparently several other enzymic activities) appear soon after changing the growth conditions [3]. These effects were minimized by quick cooling the culture to 2-4 °C in a dry ice-acetone bath [3], but as an additional cross-check, 10-ml aliquots of cultures (cells plus medium) were directly extracted, and the total PG concentrations were assayed. When the sums of PG concentrations in the cells and in the medium were much higher than their total amount determined directly, as above, the results were rejected. Values depicted in Table I were verified by this procedure.

Acknowdedgments

We wish to thank Dr. F. Dray and Dr. K. Gerozissis of the Pasteur Institute, Paris, France for providing the prostaglandin antisera and for very helpfull advices. We wish to thank also Dr. Pike of the Upjohn Company, for providing pure prostaglandin samples.

Abstract

The occurrence of prostaglandins in the protozoon *Tetrahymena pyriformis* was unequivocally demonstrated by two methods, gas liquid chromatography and radioimmunoassay. The levels of prostaglandins E_1, E_2 and F_{2a}, in pg/100-ml culture (8×10^7 cells), were found 210 ± 40, 270 ± 60 and 380 ± 160 respectively for the cell mass, and 3300 ± 200, 4000 ± 500 and 2100 ± 300 respectively for the growth medium. Cell homogenates incubated at 37°C show a rapid increase of prostaglandin concentrations, followed by gradual decrease to normal levels or below them within 3-4 min. Especially, the concentration of prostaglandin F_{2a} shows a 12-fold increase within 1 min, suggesting a very active endoperoxide cleavage under these conditions.

Περίληψη

Ύπαρξη προσταγλανδινών στην Tetrahymena pyriformis

Η ύπαρξη προσταγλανδινών στο πρωτόζωο Tetrahymena pyriformis αποδείχθηκε με δυο μεθόδους, αεριοχρωματογραφία και ραδιο-ανοσολογικές μετρήσεις (RIA). Τα επίπεδα των προσταγλανδινών E_1, E_2 και F_{2a} σε pg ανά 100 ml καλλιέργειας (8×10^7 κύτταρα) βρέθηκαν 210 ± 40, 270 ± 60 και 380 ± 160 αντίστοιχα στην κυτταρική μάζα, ενώ το μέσο ανάπτυξης βρέθηκε επίσης να περιέχει τις παραπάνω προσταγλανδίνες σε επίπεδα 3300 ± 200, 4000 ± 500 και 2100 ± 300 αντίστοιχα.

Επώαση στους 37°C ομογενοποιημένων κυττάρων προκαλεί απότομη αύξηση των συγκεντρώσεων προσταγλανδινών, που επανέρχονται σταδιακά στα κανονικά τους επίπεδα (ή και κάτω από αυτά) μέσα σε 3-4 min. Ειδικότερα, η συγκέντρωση της προσταγλανδίνης F_{2a} αυξάνεται 12 φορές μέσα σ' ένα λεπτό, πράγμα που αποτελεί ένδειξη ότι κάτω από τις συνθήκες αυτές αυξάνεται πολύ δραστικά η διάσπαση των ενδοϋπεροξειδίων.

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NOTE

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ANTITUMOR ACTIVITY OF PLATINUM (II) AND PALLADIUM (II) COMPLEXES OF S-2-AMINOETHYL-L-CYSTEINE AND S-2-AMINOETHYL-D,L-PENICILLAMINE

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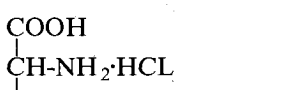
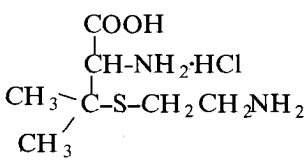
In two previous papers[1,2] we explained the synthesis and interpretation of the structure of Pt(II) and Pd(II) complexes with amino acid derivatives. The rational for the synthesis of these complexes was to elucidate as far as possible the relationship structure-biological activity and to try to find a new group of compounds with strong anticancer activity.

This communication is to report the preliminary results of the in vivo screening of the Pt(II) and Pd(II) complexes of S-2-Aminoethyl-L-Cysteine and S-2-Aminoethyl-D, L-Penicillamine hudrochloric salts





The complexes were prepared using a general method in the ratio I:I (amino acid:metal) and described in our paper[2]. The structure of the complexes was studied by elemental analysis (C, H and N), infrared and ^{1}H NMR spectra. From the spectroscopic data it was concluded that the amino acid derivatives behave as bidentate ligands, being bound to the metals via the sulphur atom and the amino group near the carboxylic group. There are also spectral evidence of a possible equilibrium between the uncoordinated carboxylic group and the electrostatic form of COO^-... M (M = Pt or Pd)[2].

The screening of the four complexes was carried out in the laboratory of the Institute Jules Bordet, Brussels, in association with the U.S. National Cancer Institute, Bethesda, Maryland.

The screening was in accordance with the screening protocol (Instruction 14. Screening Data Summary, Interpretation and Outline of Current Screen and Revisions, June 1980) for L-1210 lymphoid leukaemia or P388 lymphocytic leukaemia in male or female mice. The number of animals in each test and control group was 6 or 5. The animals were inoculated intraperitonealy with diluted ascitic

TABLE I : Summary of screening data for antitumor activity of the Pt(II) and Pd(II) complexes in mice.

Substance tested	solubility	test system	sex	dose range mg/Kg	tumor evaluation days survival contr.	test.	% T/C
(S-2-aminoethyl-L-cysteine.HCl)$PtCl_2$	Solution	L-1210	M	200	8.2	9.3	113
	»	»	M	100	8.2	9.0	109
	»	»	M	50	8.2	8.8	107
	»	»	M	25	8.2	8.3	101
	»	»	M	12,5	8.2	8.0	97
	»	»	M	6.25	8.2	7.9	96
	»	»	M	3.12	8.2	7.9	96
(S-2-aminoethyl-L-cysteine.HCl)$PdCl_2$	suspen-sion	P388	F	200	10.0	9.5	95
	»	»	F	100	10.0	9.5	95
	»	»	F	50	10.0	10.0	100
(S-2-aminoethyl-D,L-penicillamine.HCl)$PtCl_2$	Suspen-sion	L-1210	M	200	8.2	12.0	146
	»	»	M	100	8.2	10.3	125
	»	»	M	50	8.2	9.0	109
	»	»	M	25	8.2	9.0	109
	»	»	M	12,5	8.2	8.8	107
	»	»	M	6.25	8.2	8.2	100
	»	»	M	3.12	8.2	8.0	97
	suspen-sion	L-1210	F	200	8.2	16.3	198
	»	»	F	100	8.2	11.4	139
	»	»	F	50	8.2	10.8	131
	»	»	F	25	8.2	10.0	121
	solu-tion	»	F	12.5	8.2	9.4	114
	»	»	F	6.25	8.2	9.4	114
	»	»	F	3.12	8.2	8.4	102
(S-2-aminoethyl-D,L-penicillamine.HCl)$PdCl_2$	suspen-sion	P388	F	200	10.0	9.0	90
		»	F	100	10.0	10.0	100
		»	F	50	10.0	10.0	100

fluid containing 10^5 cells of the L-1210 or P388. On the next day, the animals were injected i.p. with a suspension of the compounds tested. The vehicle was Klucel for the Pd(II) complexes and saline for the Pt(II) complexes. A total of 3 injections were given at 4-day intervals. Toxicity was evaluated as survival 5 days after the first injection. Substances are judged as being active if the % T/C is 125 (or increase of lifespan of the animals by 25%).

From the data given in Table I it can be seen that, a) the Pd(II) complexes for both ligands showed no activity, b) The (S-2-aminoethyl-L-cysteine.HCl)$PtCl_2$ complex showed minimal activity for higher doses in L-1210 and no activity for lower ones, and c) the (S-2-aminoethyl-D, L-penicillamine.HCl)$PtCl_2$ is a strong antitumor agent. In male mice the % T/C for doses of 200 mg/Kg is 146 and decreases substantially for lower doses, whereas in female mice the % T/C is 198 (almost doubled the lifespan of the leukaemic mice) for the dose of 200 mg/Kg respectively.

This is, in our opinion, a very promissing result and we will continue our investigation of similar and new complexes with Pt(II) and Pd(II) and amino acid derivatives, having in mind to elucidate the connection between features of the chemical structure of the complexes and antitumor activity.

Abstract

Platinum (II) and Palladium (II) complexes of S-2-aminoethyl-L-cysteine and S-2-aminoethyl-D, L-penicillamine were screened in association with the U.S. National Cancer Institute, Bethesda, Maryland, for L-1210 lymphoid leukaemia or P388 lymphocytic leukaemia in male or female mice. One of the complexes with Pt(II) showed strong antitumor activity in female mice by doubling their lifespan. The Pd(II) complexes showed no activity.

Περίληψη

Αντικαρκινική δράση των συμπλόκων Pt(II) και Pd(II)

Σύμπλοκα Pt(II) και Pd(II) με S-2-αμινοαιθυλο-L-κυστεϊνη και S-2-αμινοαιθυλο-D, L-πενικιλλαμίνη δοκιμάσθηκαν, σε συνεργασία με το Εθνικό Ινστιτούτο Υγείας των ΗΠΑ, σε L-1210 λυμφοειδή λευχαιμία ή P388 λυμφοκυτταρική λευχαιμία σε αρσενικούς και θηλυκούς ποντικούς. Ένα από τα σύμπλοκα με Pt(II) έδειξε ισχυρή αντικαρκινική δράση σε θηλυκά ποντίκια διπλασιάζοντας τη διάρκεια ζωής τους. Τα σύμπλοκα Pd(II) δεν παρουσίασαν αντικαρκινική δράση.

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