Ichthyothereol and Its Acetate, the Active Polyacetylene Constituents of *Ichthyothere terminalis* (Spreng.) Malme, a Fish Poison from the Lower Amazon[1,2]

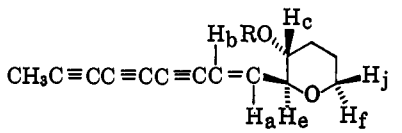
Seiva C. Cascon, Walter B. Mors, Bernard M. Tursch, Robin T. Aplin, and Lois J. Durham[3]

Contribution from the Divisão de Tecnologia Agrícola e Alimentar, Minist'rio da Agricultura, Brasil, Centro de Pesquisas de Produtos Naturais, Faculdade Nacional de Farmácia, Universidade do Brasil, Rio de Janeiro, and the Department of Chemistry, Stanford University, Stanford, California. *Received May 25, 1965*

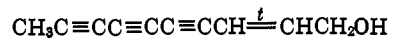
Two toxic constituents, ichthyothereol (Ia) and its acetate (Ib) have been isolated from the leaves of Ichthyothere terminalis (Family Compositae). Their structures and absolute configuration have been determined by physical methods. The results of nuclear magnetic double resonance studies and the mass spectral fragmentation of these compounds are discussed.

Ichthyothereol and its acetate have been isolated from the leaves of *Ichthyothere terminalis* (Spreng.) Malme (= *I. cunabi* Mart.), Family *Compositae* (Tribe *Heliantheae*, subtribe *Melampodinae*),[4] a small herb frequently encountered among the "campos" vegetation of Brazil and the Guyanas, and popularly known as cunabi, cunami, or cunambi. Its leaves have long been known to be used as a fish poison by the natives of the Lower Amazon Basin.[5] The peculiar custom of using the plant in baits prepared with locusts or manioc flour that are thrown into the water to be swallowed by the fish suggested that the active principle was very different from the better known fish poisons, which are mostly obtained from species of the *Leguminosae* (*Derris* spp. and related genera containing rotenoids) or from species of the *Sapindaceae* (with high saponin content), where the poison is simply diluted with water. Lacerda[6] found crude extracts of the leaves extremely poisonous not only to fish but to mammals as well. The effects in dogs were typically convulsant, similar to those of picrotoxin, indicating bulbar action. In the present work, toxicity tests were run on the fish *Lebistes reticulatus*, which reacts promptly to even minute quantities of the poison with extreme agitation and death after a few minutes. The isolation and purification was followed by such tests.

Chromatography, in the absence of light, of the petroleum ether extract of the leaves on silica gel, leads to the isolation of two active, crystalline compounds: ichthyothereol (Ia), $C_{14}H_{14}O_2$, m.p. 89–90°, $[\alpha]_D$ −44°, and its acetate (Ib), $C_{16}H_{16}O_3$, m.p. 63–65°, $[\alpha]_D$ +7°. The molecular weights as determined by their mass spectra (Figures 1 and 2) were in agreement with the analytical data. The empirical formula of ichthyothereol (Ia) was confirmed by its high-resolution mass spectrum[7] (Table II). The infrared spectra of both compounds (in chloroform solution) showed peaks at 1624 and 948 cm.$^{-1}$ indicative of a conjugated *trans* double bond and a band at 2210 cm.$^{-1}$ indicative of a disubstituted acetylene. Ichthyothereol (Ia) showed a strong hydroxyl absorption at 3600 cm.$^{-1}$, absent in the spectrum of the acetate (Ib), which shows carbonyl absorption at 1755 cm.$^{-1}$ instead. A series of bands in the region 1150–1000 cm.$^{-1}$ suggested that the second oxygen was present as an ether oxygen. The polyacetylenic nature of these two compounds, which is in keeping with their botanical origin, was demonstrated by their ultraviolet absorption spectra, which were identical with that of *trans*-dehydromatricarianol (II).[8]



Ia, R = H
Ib, R = Ac



II

N.m.r. Spectra. The n.m.r. spectrum of ichthyothereol acetate (Figure 4-1) shows the expected characteristics for structure Ib. Prominent features of the spectrum include the signals of the acetate methyl (2.04 p.p.m.), the acetylenic methyl (1.98 p.p.m.), and the two vinyl protons which appear as the AB part of an ABX pattern (6.29 and 5.80 p.p.m.), with a characteristic *trans* coupling (16.5 c.p.s.). Other assignments of chemical shifts are given in Table I along with data for ichthyothereol and the reduction product of its acetate.

Double-resonance experiments[9] summarized in Figure 4 (2, 3, 4) provide evidence for the sequence of the

(1) Preliminary communication: C. Chin, E. R. H. Jones, V. Thaller, R. T. Aplin, L. J. Durham, S. C. Cascon, W. B. Mors, and B. M. Tursch, *Chem. Commun.* (London), **8**, 152 (1965).

(2) The work at Stanford was supported in part by financial assistance from the National Institutes of Health (Grants AM-04257 and GM 11309-02). The work in Brazil was supported in part by financial assistance from the Conselho Nacional de Pesquisas, and in part from the Rockefeller Foundation.

(3) Author to whom inquires should be addressed at Stanford University.

(4) For botanical identification the authors are indebted to Dr. João Murça Pires, of the Department of Botany, University of Brasília, and to Mrs. Graziella M. Barroso, of the Botanical Garden, Rio de Janeiro.

(5) J. Baker and A. W. Eichler in "Flora Brasiliensis," C. F. Ph. Martius, Ed., Vol. 6, Munich, 1882–1884, p. 410.

(6) J. B. Lacerda, *Arch. Mus. Nac.*, **15**, 91 (1909).

(7) Determined on an Associated Electrical Industries MS 9 high-resolution mass spectrometer using a direct probe: resolution 15,000, 5% definition.

(8) J. N. Gardner, E. R. H. Jones, P. R. Leeming, and J. S. Stephenson, *J. Chem. Soc.*, 691 (1960).

(9) Double-resonance experiments were carried out on the 100-Mc. instrument using a modification of the method of Freeman and Whiffen: L. F. Johnson, "Varian Technical Information Bulletin, III", No. 3, Varian Associates, Inc., Palo Alto, Calif., 1962, p. 5; R. Freeman, *J. Mol. Phys.*, **3**, 435 (1960); R. Freeman and D. H. Whiffen, *Proc. Phys. Soc.* (London), **79**, 794 (1962).

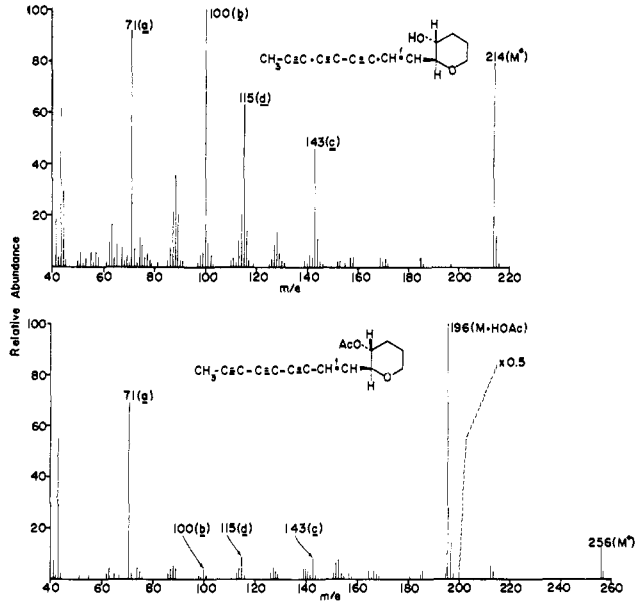


Figure 1.
Figure 2.

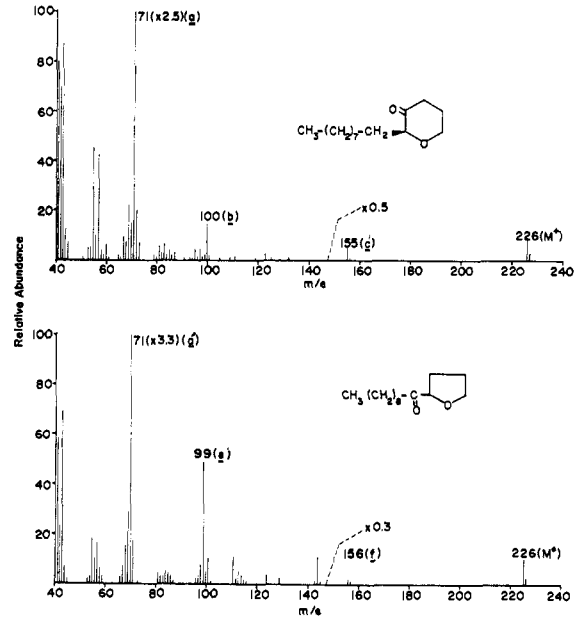


Figure 3.

coupling interactions which in turn serve to identify all of the midfield signals. These data, along with the coupling constants, establish the relative stereochemistry of the side chain and acetate groups.

Table I. Chemical Shift Assignments for Ichthyothereol and Related Compounds

Proton signal assigned to	Ichthyothereol acetate (Ib), p.p.m.	Ichthyothereol (Ia), p.p.m.	Perhydroichthyothereol acetate, p.p.m.
CH_3	1.98	1.98	0.88
H_a (*trans*)	6.29	6.50	*b*
H_b (*trans*)	5.80	5.83	*b*
H_c (ax.)	4.50	3.3*	4.55
H_d (eq.)	3.97	3.93	3.92
H_e (ax.)	3.75	3.55[a]	2.9–3.7[a]
H_f (ax.)	3.42	3.4[a]	2.9–3.7[a]
$H_{others\ on\ ring}$	1.4–2.8[a]	1.0–2.5[a]	0.7–2.5[a]
R	2.04	1.0–2.5*	2.04
Side-chain CH_2	*b*	*b*	1.28

[a] Indicates overlapping multiplets, approximate chemical shift estimated from double resonance studies of region given. [b] Indicates no such group present in compound.

The vinyl protons (a,b) in addition to their characteristic *trans* coupling of 16.5 c.p.s. are each coupled to another proton (e), the vicinal coupling (J_{ae}) being 5 c.p.s. and the allylic coupling (J_{be}) about 1.6 c.p.s. These couplings are best observed in the double-resonance experiments (Figure 4 (2 and 3)) where the appropriate splitting disappears upon irradiation of the interacting proton signal while observing H_e. The small coupling is not readily observed in the vinyl proton (H_b), presumably due to broadening of that signal by coupling to the methyl, which is slightly broadened and thus not as tall as the acetate methyl. Such long-range couplings, though small, have been reported previously.[10]

(10) E. I. Snyder and J. D. Roberts, *J. Am. Chem. Soc.*, 84, 1582 (1962), and references therein.

The proton signal at 3.75 p.p.m. (H_e) may also be shown to be coupled to the one at 4.50 p.p.m. (H_c) (Figure 4-3). The coupling constant of about 9 c.p.s. indicates an axial–axial interaction in such a six-membered ring.[11] The identity of this signal (H_c) is confirmed by its upfield shift in ichthyothereol where it appears along with the other axial protons on carbon bearing oxygen. The chemical shift of this signal (in the acetate) is only slightly affected by reduction of the conjugated side chain (see Table I, H_c).

The n.m.r. spectrum of the perhydroichthyothereol acetate still shows the three midfield proton signals and the lower field one; thus the ring system and acetate are unaffected by the reduction. The methyl signal shifts upfield and becomes a perturbed triplet on the side of an intense methylene signal (centered at 1.28 p.p.m.) with the characteristic shape of a long, straight-chain hydrocarbon.

Since the n.m.r. evidence indicates that H_c and H_e bear an axial–axial relationship, the acetylenic side chain and the acetate (or hydroxyl in Ia) must be equatorial, thus establishing the relative stereochemistry. Oxidation of perhydroichthyothereol with Jones reagent[12] gave the ketone III, the optical rotatory dispersion curve of which showed a negative Cotton effect. No correlation of O.R.D. data for substituted tetrahydropyran-3-ones has yet appeared in the literature; therefore, the existing data are insufficient to ascertain the absolute configuration of the molecule. If, however, it is assumed that the "octant rule"[13] applies equally to tetrahydropyran-3-ones as to cyclohexanones, then III should exhibit a negative Cotton effect whereas

(11) Although axial–axial coupling constants are generally observed to be 8–14 c.p.s. and axial–equatorial ones 1–7 c.p.s., addition of electronegative groups, such as oxygen of ether or acetate, tend to decrease these values making the 9-c.p.s. value a definite axial–axial one here. For a discussion of these factors see N.S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, Chapter III, pp. 49–61.

(12) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(13) W. Moffitt, A. Moscowitz, R. B. Woodward, W. Klyne, and C. Djerassi, *J. Am. Chem. Soc.*, 83, 4013 (1961).

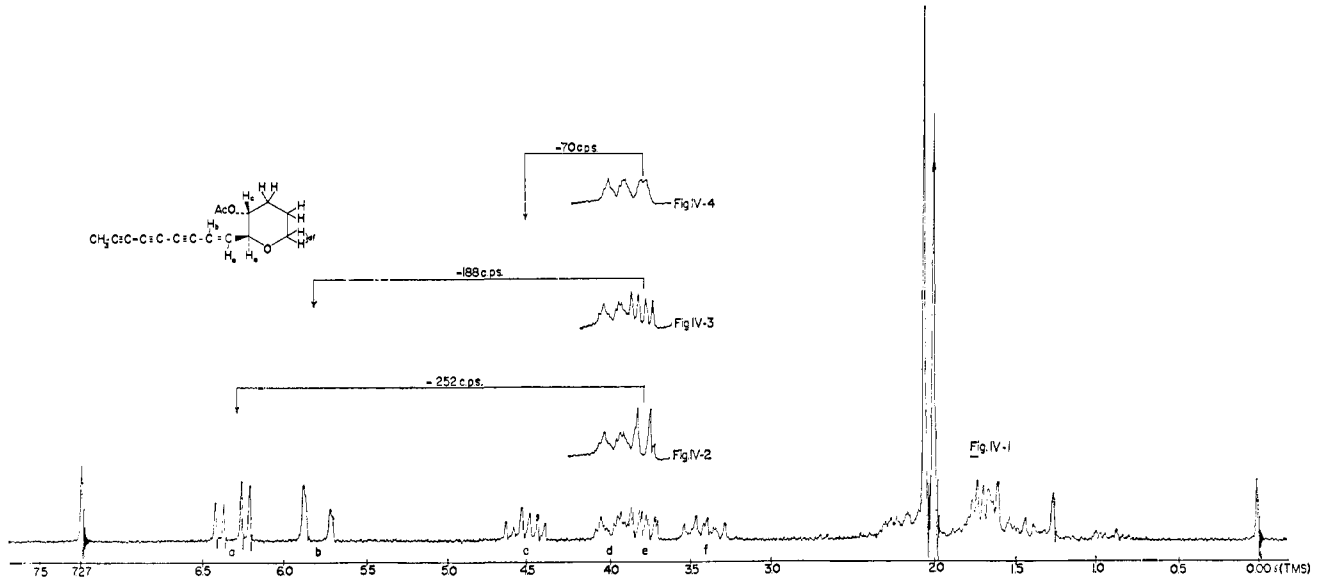
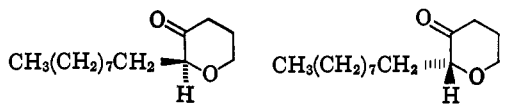


Figure 4. Ichthyothereol acetate nuclear magnetic double-resonance studies.

its enantiomer IV should have a positive Cotton effect. Since the relative stereochemistry of the alkyl side chain and the hydroxyl, or acetate, have been established by the n.m.r. spectrum, the absolute configuration of ichthyothereol, derived from the negative Cotton effect observed for the ketone III, would be that shown in Ia.



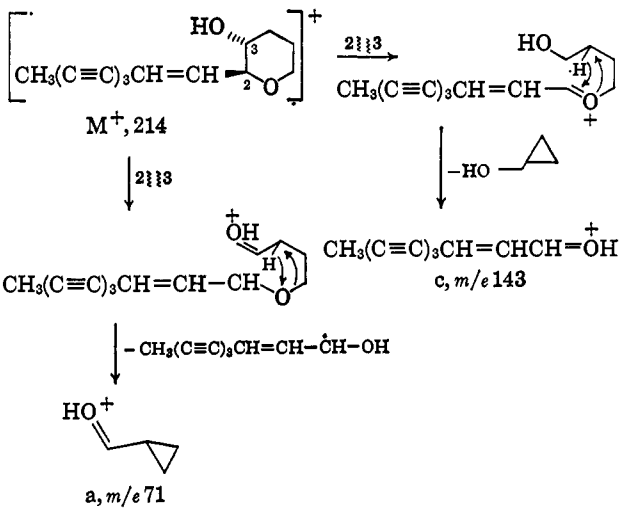
Mass Spectra. The mass spectra of ichthyothereol (Ia, Figure 1), its acetate (Ib, Figure 2), and the ketone (III, Figure 3, upper) have three common peaks: a, *m/e* 71; b, *m/e* 100; and c, *m/e* 143 (Figures 1 and 2); c′, *m/e* 155 (Figure 3, upper). A further peak (d) *m/e* 115 (Figures 1 and 2) is of interest as an intense metastable peak *m/e* 92.6 (calcd. 92.6) shows that it is formed by a one-step process from the *m/e* 143 peak. The composition (Table II) of the peaks (a, b, c, and d) in Figure 1 were established by the high-resolution spectrum.[7]

Table II. High-Resolution Data for Ichthyothereol

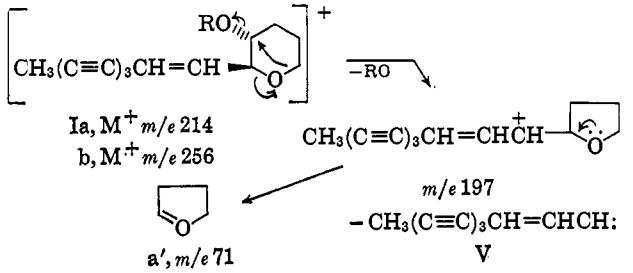
Peak					Mass[b]	
m/e[a]	%	Compn.		Ratio	Found[c]	Calcd.
214 P	80	$C_{14}H_{14}O_2$			214.0994	214.099373
143 D	46	$C_{10}H_7O$			143.0505	143.049687
			$C_{11}H_{11}$	20:1	143.0873	143.086071
115	64		C_9H_7		115.0549	115.054773
100 D	100	$C_5H_8O_2$	C_8H_4	5:1	100.0520	100.052426
					100.0308	100.031299
71	93	C_4H_7O			71.0497	71.049687

[a] P = parent; D = doublet. [b] ^{12}C mass scale. [c] Values ±0.0005 above *m/e* 99 and ±0.00005 below *m/e* 99.

The origin of a, *m/e* 71 and the M − 71 peak (c), *m/e* 143 can be rationalized as indicated as follows. It is also possible that part of the *m/e* 71 peak arises from the M − 17, or M − 43 (Figure 2), peak by ex-

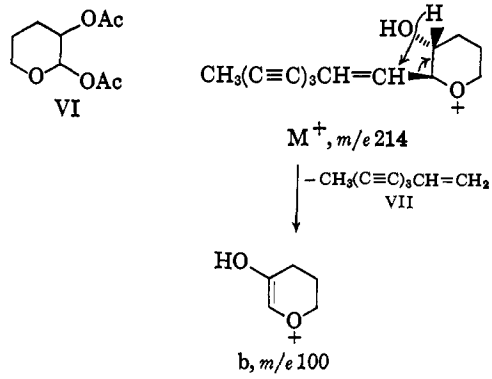


pulsion of a conjugated carbene (V), to give the isomeric oxonium ion a′. This type of cleavage has been



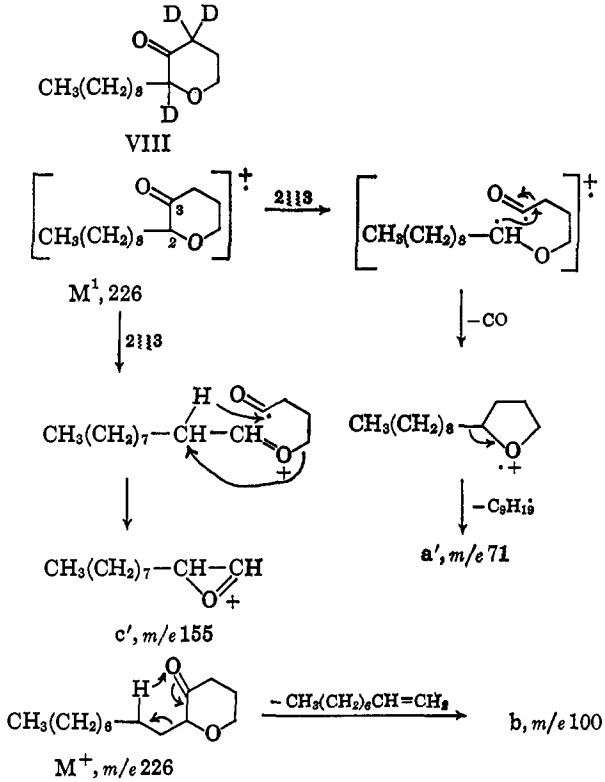
evoked to explain the origin of the base peak, *m/e* 71, in the spectrum of the tetrahydropyrane (IV),[14] and may well represent the major mode of formation of the *m/e* 71 peak in the spectrum of the acetate (Figure 2). The origin of the species b, *m/e* 100, can be represented as shown, the driving force for the cleavage being the stability of the neutral species (VII). These peaks (b and c) are rather weak in the spectrum of the acetate (Ib, Figure 2) and probably arise from the weak M − 42 (CH_2CO, *m/e* 172) peak. The loss of carbon monox-

(14) M. Vengopalan and C. P. Anderson, *Chem. Ind.* (London), 37 (1964).

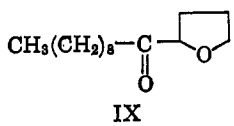


ide (CO) from the species c, *m/e* 143, to give d $C_9H_7^+$, *m/e* 115, is extremely difficult to rationalize. The mass spectra of polyacetylenes in general are the subject of a current study.

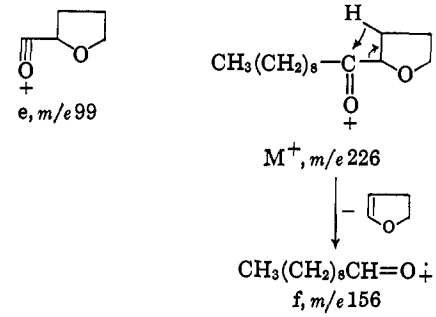
In the mass spectrum of the ketone (III, Figure 3, upper) the base peak a′ is still at *m/e* 71; the peaks b, *m/e* 100 and c′, *m/e* 155 (M − 71), although still present are very weak. In the spectrum of the D_3-ketone (VIII) a′ is moved to *m/e* 74, b to *m/e* 103, and c to *m/e* 156. On the basis of these shifts the origin of the peaks a, b, and c can be rationalized as indicated.



From its mass spectrum (Figure 3, upper) the ketone III was originally assigned the isomeric structure IX. However, the spectrum of this ketone[15] (Figure 3, lower)



is different from that of III. The base peak *m/e* 71 is the oxonium ion a′ which is typical for α-substituted furans.[16] Cleavage β to the furan gives the species e, *m/e* 99. The M − 70 peak f, *m/e* 156, can be rationalized as indicated.



In the light of the mass spectra discussed above, the appearance of a very intense *m/e* 71 peak points to the need for caution in using this peak as being characteristic of the presence of an α-substituted tetrahydrofuran.

Experimental Section[17]

Isolation of Ichthyothereol (Ia) and Its Acetate (Ib). Dried and ground leaves (760 g.) were extracted with petroleum ether (b.p. 60–80°) in a Soxhlet apparatus, protected from excessive illumination. The extract was concentrated and chromatographed on a column of 700 g. of silica gel (Merck). Elution was done first with 1.5 l. of petroleum ether (b.p. 60–80°), followed by a gradient between 3 l. of petroleum ether and 6 l. of benzene, and finally chloroform. Fractions (250 ml. each) were collected. Ichthyothereol acetate was present in fractions 33–41; the free alcohol in fractions 49–60.

Purification of Ichthyothereol Acetate (Ib). Fractions 33–41 were combined and placed in a freezer. An amorphous, white, waxy material separated out first. This appeared to be a saturated aliphatic alcohol and was not further investigated. From the filtrate Ib crystallized later. Several recrystallizations were done with petroleum ether (b.p. 30–60°) at 0° in order to separate the acetate from a red oil, seemingly its oxidation product when in solution. When exposed to light in the solid state, the substance turns violet and becomes insoluble in petroleum ether. It is fairly stable at 0° under nitrogen or immersed in a small amount of solvent. The compound crystallized as white needles: m.p. 63–65°; $[\alpha]_D$ +7°; λ_{max} 325 mμ (ε 15.1 × 10³), 308 (22.4 × 10³), 289 (16.9 × 10³), 272.5 (8.9 × 10³), 257 (6.2 × 10³), 245 (60.0 × 10³), 232 (52.0 × 10³), and 210 (33.8 × 10³). *Anal.* Calcd. for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.31; H, 6.52.

Purification of Ichthyothereol (Ia). Ichthyothereol from fractions 49–60 was purified by several recrystallizations from petroleum ether (b.p. 60–80°), m.p. 89–90°, $[\alpha]_D$ −44°. The infrared spectrum showed λ_{max} 3650 and 3500 (OH), 2250 (C≡C), 1640 (CH=CH,

(15) Sample kindly provided by Professor Sir Ewart R. H. Jones.

(16) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, Calif., 1964, p. 270.

(17) Thanks are due to Mr. Nilo Tomás da Silva, Instituto de Pesquisas e Experimentação Agropecurárias do Norte, Belém, for repeated collections of the plant material. The n.m.r. spectra of deuteriochloroform solutions of ichthyothereol (Ia) and its acetate (Ib) were obtained at 60 and 100 Mc. using Varian A-60 and HR-100 n.m.r. spectrometers. The chemical shifts are reported in parts per million (p.p.m.) relative to internal tetramethylsilane taken as zero. Rotations were carried out in chloroform solution at room temperature.

trans), and 1085 cm.$^{-1}$ (C—O—C). The ultraviolet spectrum is practically identical with that of Ib. *Anal.* Calcd. for $C_{14}H_{14}O_2$: C, 78.48; H, 6.59. Found: C, 78.79; H, 6.70. The compound is more stable than its acetate; when exposed to light it turns brown.

Hydrogenation of Ichthyothereol (Ia). Ichthyothereol (50 mg.) was hydrogenated with 25 mg. of 12% palladium-on-charcoal catalyst, in tetrahydrofurane. Purification was accomplished on a 5-g. silica gel column, eluting by means of a gradient between 50 ml. of benzene and 50 ml. of ethyl ether. Fractions of 5 ml. each were collected. Pure perhydroichthyothereol was present in fractions 6 and 7, as a colorless oil, showing no infrared maxima indicative of unsaturation.

Hydrogenation of Ichthyothereol Acetate (Ib). Hydrogenation was performed in the same way as in the case of Ia. The crude hydrogenated product (100 mg.) was purified on a 10-g. silica gel column, eluting with a gradient between 150 ml. of petroleum ether (b.p. 30–60°) and 150 ml. of benzene. The pure product was present in fractions 16 and 17 (colorless oil, showing no infrared maxima indicative of unsaturation).

Preparation of Ketone III. Perhydroichthyothereol (15 mg.) in acetone (10 ml.) was oxidated with Jones reagent.[12] Isolation via ether gave the crude ketone which was purified by a short-path distillation (bath temp. 100° at 0.07 mm.). This afforded the ketone III as a viscous oil, $[\alpha]_D$ −28°, O.R.D. in methanol, $[\phi]_{320}$ −777°, $[\phi]_{277}$ +643°; amplitude, a = 14.

Interaction and Association of Bases and Nucleosides in Aqueous Solutions. IV. Proton Magnetic Resonance Studies of the Association of Pyrimidine Nucleosides and Their Interactions with Purine[1b]

M. P. Schweizer,[2a] Sunney I. Chan,[2b] and P. O. P. Ts'o[2a]

Contribution from the Department of Radiological Sciences, The Johns Hopkins University, Baltimore, Maryland, and the Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California.[1a] *Received May 26, 1965*

The mode of self-association of purines has been elucidated by p.m.r. as reported previously. Association of purines is manifested by ring-current magnetic anisotropy effects which result in shifts of the resonance lines to higher field with increasing concentration. Such shifts were not found in the association of nonaromatic uridine, cytidine, and thymidine in solutions or mixtures. However, protons of these pyrimidine nucleosides are shifted upfield with increasing purine concentration; for example, over the purine concentration range 0.0–1.0 m (D_2O, 35°), H-6 and CH_3 of thymidine are shifted by 0.40 and 0.34 p.p.m., respectively. This effect falls off progressively as the proton distance from the ring increases, i.e., H-1′ by 0.31 and H-5′ by 0.08–0.10 p.p.m. The effect of purine on deoxyribose is negligible. Purine-induced changes in the thymidine sugar patterns are also observed. All these data strongly suggest that the purine–nucleoside interaction takes place at the pyrimidine base through vertical ring stacking. The strong self-association of purine in stacks can be reduced by addition of these nonaromatic pyrimidine nucleosides, presumably by insertion or destacking.

Introduction

In the continuing quest for knowledge concerning the molecular basis of nucleic acid structure and interaction, we have recently initiated a program to study systematically the association of bases and nucleosides in aqueous solution. The results from these initial efforts have been reported in the preceding papers of this series.[3–5] The information obtained from the investigation of these monomer systems has provided new insights into possible interactions of the monomeric units in the polymeric nucleic acid state.

Insight into the interactions of the monomers has come from two sources. From osmotic studies[3,4] it was demonstrated that these bases and nucleosides exhibit a high degree of association in neutral aqueous media. It was further established that interactions between purine bases and nucleosides were more favorable than cross-interactions between purine and pyrimidine bases and nucleosides, and that these latter interactions were in turn more favorable than interactions among the pyrimidine bases and nucleosides themselves. From proton magnetic resonance studies[5,6] the mechanism of the self-association of purine and 6-

(1) (a) Contribution No. 3253. Paper presented in part at the Ninth Annual Meeting of the Biophysical Society, San Francisco, Calif., Feb. 1965. (b) This work was supported in part by Grants No. GM 10802-03 and GM-K3-16-704 from the National Institutes of Health, U. S. Public Health Service, and by Grant No. GB-767 from the National Science Foundation.

(2) (a) Department of Radiological Sciences, The Johns Hopkins University; (b) Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, Calif.

(3) P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, *J. Am. Chem. Soc.*, **85**, 1289 (1963).

(4) P. O. P. Ts'o and S. I. Chan, *ibid.*, **86**, 4176 (1964).

(5) S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, *ibid.*, **86**, 4182 (1964).

(6) Similar experimental findings for purine and nucleotides have been independently observed by Jardetzky. See O. Jardetzky, *Biopolymers Symp.*, **No. 1**, 501 (1964).