mann methine,[7] $C_{20}H_{23}O_2N$, m.p. 102–103°, mass spectral peaks at *m/e* 309, *m/e* 251, *m/e* 58. Positive identification was achieved *via* the hydriodide, $C_{19}H_{21}O_2N \cdot HI$, m.p. 238–240°, which was directly compared with 3,6-dimethoxyaporphine hydriodide prepared from an authentic sample of (+)-3-hydroxy-6-methoxyaporphine.[8,9]

Synthesis of thalicarpine was accomplished by modified Ullmann condensation of (−)-6′-bromolaudanosine (V) with isocorydine (VI).[10] Since practical total syntheses of laudanosine[11] and of isocorydine[12] had previously been accomplished, the condensation reaction constituted a total synthesis of thalicarpine. Furthermore, since the absolute configurations of (−)-laudanosine[13] and of isocorydine[13–16] had been elucidated earlier, the synthesis served also to establish the absolute configuration of thalicarpine.[17,18]

(8) We thank Professor M. Tomita, Kyoto University, for an authentic sample of 3-hydroxy-6-methoxyaporphine hydrochloride.

(9) The hydrogenolysis of the methoxy group at C-5 of the aporphine residue of thalicarpine finds close analogy in a similar cleavage of O-methyldomesticine described in ref. 7.

(10) We thank Dr. R. H. F. Manske, Dominion Rubber Co. Ltd., Guelph, Ontario, for a generous gift of isocorydine.

(11) A. Pictet and M. Finkelstein, *Ber.*, **42**, 1979 (1909).

(12) I. Kikkawa, *J. Pharm. Soc. Japan*, **78**, 1006 (1958).

(13) H. Corrodi and E. Hardegger, *Helv. Chim. Acta*, **39**, 889 (1956).

(14) E. Späth and F. Berger, *Ber.*, **64**, 2038 (1931).

(15) W. A. Ayer and W. I. Taylor, *J. Chem. Soc.*, 472 (1956).

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(17) Satisfactory analyses have been obtained for all compounds with cited empirical formulas. We thank Mr. Joseph Alicino, Metuchen, N. J., and Dr. S. M. Nagy, Cambridge, Mass., for the analyses.

(18) This investigation was supported in part by research grants from the National Institutes of Health (H-2952 and CY-4500).

(19) Recipient of the 1962 Lunsford Richardson Pharmacy Award for a paper including part of this work.

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON 6, WISCONSIN

S. MORRIS KUPCHAN
NAOKATA YOKOYAMA[19]

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THE ISOLATION OF A PENTACYCLIC TRITERPENOID ALCOHOL FROM A PROTOZOAN[1]

Sir:

We have isolated a saturated pentacyclic triterpenoid alcohol as the principal component of the non-saponifiable lipid fraction from the ciliated protozoan, *Tetrahymena pyriformis*. We believe this constitutes the first known case in which this type of compound has been obtained from an organism of the animal kingdom; pentacyclic triterpenoids have been found previously only in plants.[2]

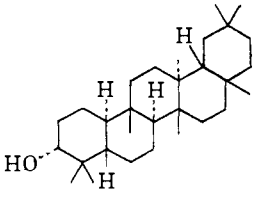
This alcohol, for which we propose the name tetrahymanol, was obtained as a white crystalline solid with m.p.[3] 312.5–314.5° (*Anal.*[4] Calcd. for $C_{30}H_{52}O$: C, 84.04; H, 12.23. Found: C, 84.04, 83.89; H, 12.09, 12.20)[5] by saponification of the material extracted from the lyophilized organisms[6] with 30–60° petroleum ether in a Soxhlet apparatus followed by purification of the crude alcohol by chromatography on alumina, recrystallization from methanol and sublimation at reduced pressure. The molecular formula of tetrahymanol was established unambiguously as $C_{30}H_{52}O$ by mass spectral studies[7] of the parent alcohol and also of its derivatives, tetrahymanyl acetate, m.p. 303–305° (*Anal.* Calcd. for $C_{32}H_{54}O_2$: C, 81.64; H, 11.56. Found: C, 81.66, 81.43; H, 11.46, 11.27), and tetrahymanone,[8] m.p. 289–290° (*Anal.* Calcd. for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.39, 84.25; H, 11.83, 11.62).

The 60-Mc. proton n.m.r. spectra[9] of tetrahymanol and tetrahymanyl acetate each show four sharp peaks characteristic of triterpenoid methyl groups[10] at $\tau >$ 9.03 with area corresponding to eight methyls (tetrahymanol: 46, 49, 51 and 58 c.p.s. with relative areas 1:3:1:3; tetrahymanyl acetate: 47.5, 49, 50.5 and 58 c.p.s. with relative areas 1:1:4:2). The methyl peaks in the 100-Mc. spectrum[9] of the acetate are observed at 79, 81.5, 84 and 96.5 c.p.s.; the increase by the factor 100/60 in all these peak separations in the 100-Mc. spectrum compared with those in the 60-Mc. spectrum indicates the absence of strong spin–spin coupling of any methyl protons with other protons in the molecule and thus demonstrates that no methyl group is attached to a carbon which also bears a hydrogen. Of the various triterpenoid skeletons known to occur naturally, only the oleanane skeleton or certain skeletons related to that of oleanane by backbone rearrangements could be consistent with these n.m.r. results.

The n.m.r. spectrum of tetrahymanol exhibits a multiplet peak centered at τ = 6.75 with area corresponding to one proton; the spectrum of tetrahymanyl acetate shows a similar multiplet peak centered at τ = 5.50. Such peaks are characteristic[10] of protons alpha to equatorial (as opposed to axial) hydroxyl groups or acetoxy groups, respectively. The lack of absorption peaks at lower field strengths than these multiplets at τ = 6.75 and 5.50 in the two spectra demonstrates the absence of olefinic protons in these molecules.

From the structural assignments given by Lehn[10a,b] for the methyl peaks in the 60-Mc. n.m.r. spectra of triterpenoids and from additional data[11] it can be concluded that the C-4 *gem*-dimethyl group in a typical triterpenoid having no unsaturation close to C-4 gives rise to two peaks near 46 and 58 c.p.s. if there is an equatorial hydroxyl group at C-3, but gives rise to two superimposed peaks near 51 c.p.s. if there is an equatorial acetoxy group at C-3. On this basis the 60-Mc. spectra of tetrahymanol and its acetate indicate that tetrahymanol has a 3-hydroxy-4,4-dimethyl configuration.

We suggest tentatively that tetrahymanol has the structure[12] indicated below.



(1) This work is supported by the Office of Naval Research, Contract Nonr-2829(02).

(2) J. Simonsen and W. C. J. Ross, "The Terpenes," Vol. IV, Cambridge University Press, London, 1957; P. de Mayo, "The Higher Terpenoids," Interscience Publishers, Inc., New York, N. Y., 1959.

(3) All melting points were measured in evacuated capillaries using a heated metal block and are uncorrected.

(4) All elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

(5) A sample of tetrahymanol with m.p. 309–312° was isolated from *Tetrahymena pyriformis* and reported erroneously to be isomeric with cholesterol by C. M. McKee, J. D. Dutcher, V. Groupé and M. Moore, *Proc. Soc. Exp. Biol. Med.*, **65**, 326 (1947).

(6) Facilities for large-scale incubations of the protozoan were generously made available by Dr. William Charney, Schering Corporation, Union, N. J.

(7) Mass spectra were obtained through the courtesy of Dr. J. G. Bendoraitis, Socony Mobil Oil Co., Inc., Paulsboro, N. J.

(8) Tetrahymanone was prepared by oxidation of tetrahymanol with chromium trioxide in pyridine at room temperature.

(9) N.m.r. spectra were determined in deuteriochloroform solution by Varian Associates, Palo Alto, Calif.; peak positions given in c.p.s. refer to downfield shifts from tetramethylsilane as an internal standard.

(10) (a) J.-M. Lehn and G. Ourisson, *Bull. Soc. chim. France*, 1137 (1962); (b) J.-M. Lehn, *ibid.*, 1832 (1962); (c) M. Shamma, R. E. Glick and R. O. Mumma, *J. Org. Chem.*, **27**, 4512 (1962); (d) R. O. Mumma, Ph.D. Thesis, The Pennsylvania State University, 1960.

(11) J. N. Shoolery and M. T. Rogers, *J. Am. Chem. Soc.*, **80**, 5121 (1958); R. F. Zürcher, *Helv. Chim. Acta*, **44**, 1380 (1961).

(12) 5β-Glutinan-3α-ol.

This structure is reasonable biosynthetically[13]; triterpenoids with similar skeletons have been isolated from various plants.[14]

In accord with this tentative structural formulation, tetrahymanone has a positive carbonyl n → π* Cotton effect centered at 290 mμ with molecular amplitude[15] +18 (R.D. in dioxane (*c* 0.14), 27°; $[\alpha]_{450}$ +73°, $[\alpha]_{313}$ +389°, $[\alpha]_{308}$ +375°, $[\alpha]_{306}$ +376°, $[\alpha]_{273}$ −38.6°, $[\alpha]_{240}$ +236°).[16] Thus, the Cotton effect curves[17] for 4,4-dimethyl-19-nordihydrotestosterone and 4,4,17α-trimethyl-19-nordihydrotestosterone, two compounds whose configuration in the vicinity of the carbonyl group is essentially the mirror image of that we propose for tetrahymanone, have molecular amplitudes of −20 and −15, respectively.[18]

We have nearly completed the rigorous establishment of the structure of tetrahymanol by an X-ray crystallographic study[19] of its *p*-bromobenzoate ester, m.p. 297–298.5° (*Anal.* Calcd. for $C_{37}H_{55}BrO_2$: C, 72.64; H, 9.06; Br, 13.06. Found: C, 72.63, 72.49; H, 9.12, 8.95; Br, 13.34, 13.16). This crystal is orthorhombic with space group C_{222}; the unit cell contains four molecules and has dimensions a = 13.06, b = 6.33 and c = 38.06 Å. The shortness of the b dimension shows that the tetrahymanol skeleton is roughly flat and fully extended in accord with predictions made from consideration of a molecular model of the proposed structure in which non-chair conformations for the D and E rings appear to be favorable.

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(14) J. M. Beaton, F. S. Spring, R. Stevenson and J. L. Stewart, *Tetrahedron*, **2**, 246 (1958); K. Kimura, Y. Hashimoto and I. Agata, *Chem. Pharm. Bull.* (Tokyo), **8**, 1145 (1960); H. R. Arthur and W. H. Hui, *J. Chem. Soc.*, 551 (1961).

(15) This term is defined by N. L. Allinger and M. A. DaRooge, *J. Am. Chem. Soc.*, **84**, 4561 (1962), and by C. Djerassi and W. Klyne, *J. Chem. Soc.*, 4929 (1962).

(16) These rotatory dispersion measurements were made in the laboratories of Professor Kurt Mislow, New York University.

(17) C. Djerassi, O. Halpern, V. Halpern and B. Riniker, *J. Am. Chem. Soc.*, **80**, 4001 (1958).

(18) Since the testosterone derivatives were studied[17] in methanol solution the close agreement in absolute magnitude between these amplitudes and the amplitude for tetrahymanone[16] may be somewhat fortuitous.

(19) In collaboration with Professor T. H. Doyne, Villanova University.

FRANK B. MALLORY
BRYN MAWR COLLEGE JANICE T. GORDON
BRYN MAWR, PENNSYLVANIA ROBERT L. CONNER

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THE STEPWISE SYNTHESIS OF RIBO-OLIGONUCLEOTIDES CONTAINING C_3'–C_5' INTERNUCLEOTIDIC LINKAGES[1]

Sir:

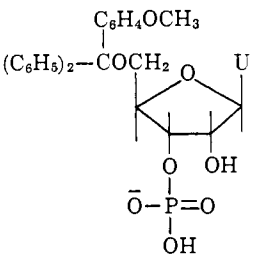
2′-*O*-Acetylribonucleoside-3′ phosphates are the key intermediates in the recently described approach to the synthesis of the C_3'–C_5' inter-ribonucleotidic linkage[2] and a method has been developed for the direct preparation of these derivatives in quantitative yield from the parent nucleotides.[2,3] In further work, this approach is being incorporated into schemes for the stepwise synthesis of ribopolynucleotides. The present communication records results of the initial phase of this work which has led to the syntheses of the two trinucleotides, adenylyl-(3′→5′)-uridylyl-(3′→5′)-uridine and uridylyl-(3′→5′)-uridylyl-(3′→5′)-uridine.

(1) This work has been supported by grants from the National Cancer Institute of the National Institutes of Health, the National Science Foundation and the Life Insurance Medical Research Fund, New York, N. Y.

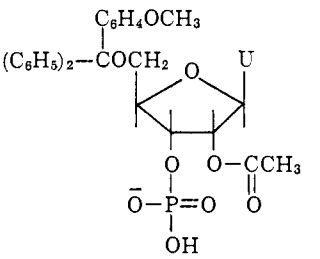
(2) D. H. Rammler and H. G. Khorana, *Biochem. Biophys. Res. Commun.*, **7**, 147 (1962); **8**, 61 (1962); D. H. Rammler, Y. Lapidot and H. G. Khorana, *J. Am. Chem. Soc.*, in press.

(3) Y. Lapidot and H. G. Khorana, *Chem. Ind.* (London), 166 (1963).

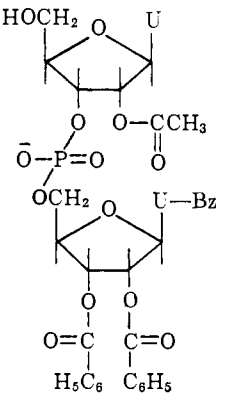
5′-*O*-monomethoxytrityluridine-3′ phosphate (I) was prepared by the reaction of pyridinium uridine-3′ phosphate with monomethoxytrityl[4] chloride (3 molar equiv.) in pyridine for 6 hr. ar room temperature and was purified by chromatography on a DEAE-cellulose (carbonate) column (isolated yield, 70%). Acetylation of I with acetic anhydride in the presence of an excess of tetraethylammonium acetate[3] gave quantitatively the 2′-*O*-acetyl derivative (II) which was obtained as a white amorphous powder (pyridine salt) after precipitation from a mixture of pyridine and an excess of ether. The condensation of II (0.1 mmole) and *N*,2′,3′-tribenzoyluridine[5] (0.245 mmole) in dry pyridine in the presence of dicyclohexylcarbodiimide (DCC) followed by a work-up including an acidic treatment to remove the methoxytrityl group gave III as the major product which was purified by partition



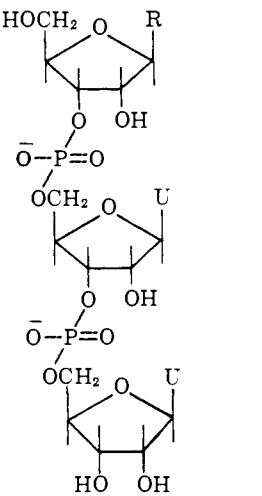
I



II



III; U-Bz = N-Benzoyluracil



IV; R = uracil or adenine
I-IV; U = uracil

TABLE I

PAPER CHROMATOGRAPHY OF DIFFERENT COMPOUNDS

Solvent A; isopropyl alcohol–concentrated ammonia–water (7:1:2)

Solvent B; *n*-butyl alcohol–acetic acid–water (5:2:3). Paper chromatography was performed using Whatman paper No. 1 by the descending technique.

Compound	R_f solvent A	R_f solvent B
Uridine-3′phosphate	0.12	0.23
5′-*O*-monomethoxytrityluridine-3′ phosphate	.52	
Uridylyl-(3′ → 5′)-uridine	.19	.09
2′-*O*-Acetyluridylyl-(3′→5′)-N,2′,3′-tribenzoyluridine (III)		.70
Uridylyl-(3′ → 5′)-uridylyl-(3′ → 5′)-uridine	.065	.03
Adenylyl-(3′→5′)-uridylyl-(3′→5′)-uridine	.065	.027

(4) M. Smith, D. H. Rammler, I. H. Goldberg and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 430 (1962). Monomethoxytrityl is abbreviation for *p*-anisyldiphenylmethyl.

(5) M.p. 174–176°. Elemental analysis and other properties clearly show one benzoyl group on the uracil ring. This group is tentatively placed on N-1 position. D. H. Rammler, private communication, and R. Lohrmann, unpublished work from this Laboratory.