reversion in the presence of maleic anhydride-2,3-C^{14}; and (2) by determining an approximate equilibrium constant [K = 1.7 M = (maleic anhydride)(2-methylfuran)/(adduct)] from the integrated n.m.r. spectra of reactants and product in CCl_4. Column 4 of Table II gives the % remaining adduct resulting from readdition as calculated from the equilibrium constant. Column 6, alternatively, gives the % reformed adduct as determined by C^{14}-incorporation. Here, one assumes that return only occurs during work up (*i.e.*, concentration after decomposition has been quenched). The specific activity of added maleic anhydride-C^{14} decreases as adduct-decomposition progresses Therefore return of a maleic anhydride-C^{14}-molecule after 80% reaction is accompanied with more return of natural anhydride than one returning after 30% reaction. Hence the figures in column 6, Table II, represent the *maximum* possible return; the true degree of reversibility is somewhere between the values given in columns 4 and 6. Since there was no initial maleic anhydride present, the amount of return, in the runs of Table I, is less than the figures of Table II.

For a two-step adduct-decomposition, k_V/k_{IV} was assumed to be 1.15. One might expect the reciprocal, 0.87, for the isotope effect of the reverse reaction. The ratio, [IV]/[V], from return should be independent of extent of return and equal to 1.15. If 15% of the isolated adduct comes *via* return then the observed effect would have led to $0.15[(IV/V)_{returned} = 1.15] + 0.85[(IV/V)_{unreacted} \cong 1.3] = 1.28$. Therefore, the small amount of adduct that comes from readdition during decomposition of the adduct does not affect the conclusion that both a and b are breaking simultaneously in the slow step. From the principle of microscopic reversibility, the formation of this Diels–Alder adduct involves forming bonds a and b simultaneously.[8]

Acknowledgment.—We acknowledge, with appreciation, the discussions with and help given by Dr. Gerald Dudek, Harvard University, in the n.m.r. studies.

(8) For a discussion of this problem see: A. Wasserman, *J. Chem. Soc.*, 612 (1942); C. Walling and J. Peisach, *J. Am. Chem. Soc.*, **80**, 5819 (1958); R. B. Woodward and T. J. Katz, *Tetrahedron*, **5**, 70 (1959); J. A. Berson, A. Remanick and W. A. Mueller, *J. Am. Chem.Soc.*, **82**, 5501 (1960); J. A. Berson and W. A. Mueller, *ibid.*, **83**, 4940 (1961), **83**, 4947 (1961); M. S. Newman, *J. Org. Chem.*, **26**, 2630 (1961); M. G. Ettlinger and E. S. Lewis, *Texas J. Sci.*, **14**, 58 (1962); J. Sauer, D. Lang and A. Mielert, *Angew. Chem. Intern. Ed. Engl.*, **1**, 268 (1962); R. P. Lutz and J. D. Roberts, *J. Am. Chem. Soc.*, **83**, 2198 (1961).

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THE STRUCTURE, CONFIGURATION AND SYNTHESIS OF THALICARPINE, A NOVEL DIMERIC APORPHINE-BENZYLISOQUINOLINE ALKALOID[1]

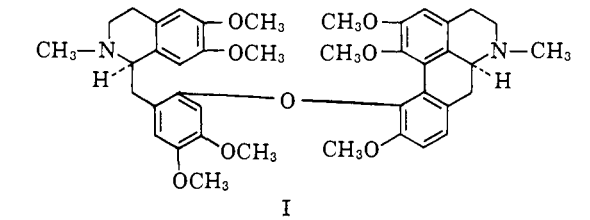
Sir:

Thalicarpine is a hypotensive alkaloid from *Thalictrum dasycarpum* Fisch. and Lall, and its isolation and preliminary characterization have recently been reported.[2] The elucidation of structure and absolute configuration I and the total synthesis of thalicarpine are reported herewith. Thalicarpine represents a novel type of alkaloid; it appears to be the first recognized dimeric alkaloid which contains an aporphine moiety.

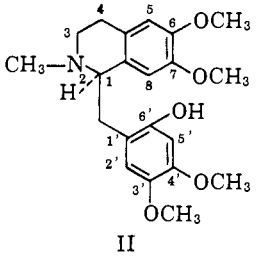
Thalicarpine (I), $C_{41}H_{48}O_8N_2$, m.p. 160–161°, $[\alpha]^{25}D$ +89°,[3] shows λ_{max} 282 mμ (ε 17,000), 302 mμ (ε 13,000) and n.m.r. peaks[4] 7.55, 7.52 (6H, two NCH_3), 6.40, 6.29, 6.21, 6.19, 6.17, 6.09, 6.05 (21H, seven OCH_3), 3.79, 3.47, 3.40, 3.37, 3.32, 1.77 τ (7H, aromatic). Hofmann degradation[2] yielded a methine which was characterized as the dimethiodide, $C_{45}H_{58}O_8N_2I_2 \cdot H_2O$, m.p. 275–276°. A second Hofmann degradation yielded the *des*-N-methine,[2] $C_{39}H_{38}O_8$, m.p. 170–172°.

Sodium in liquid ammonia reduction of I afforded (−)-6′-hydroxylaudanosine (II) and (+)-3,6-dimethoxyaporphine (III), both in amorphous form. II was characterized as the hydriodide, $C_{21}H_{27}O_5N \cdot HI \cdot H_2O$, m.p. 184–186°, $[\alpha]^{26}D$ −71° (methanol), and the O-methylmethiodide, $C_{23}H_{32}O_5NI$, m.p. 223–224°, $[\alpha]^{23}D$ +109°. Hofmann degradation of the methiodide gave a methyl methine (IVa), $C_{23}H_{31}O_5N$, m.p. 125–127°, $[\alpha]^{28}D$ ±0°, 282 mμ (ε 19,000), 343 mμ (ε 30,500), mass spectral peaks at *m/e* 401, *m/e* 58.[5] To locate the phenolic hydroxyl group, II was converted to the O-ethylethiodide, which upon Hofmann degradation yielded IVb, $C_{25}H_{35}O_5N$, m.p. 88–89°, $[\alpha]^{27}D$ ±0°; λ_{max} 290 mμ (ε 12,000), 336 mμ (ε 12,600). The methiodide of IVb ($C_{26}H_{38}O_5NI$, m.p. 201–202°) was treated with methanolic alkali to yield the *des*-N-methine, $C_{22}H_{26}O_5$, m.p. 129–130°. Oxidation of the *des*-N-methine with potassium permanganate gave 2-ethoxy-4,5-dimethoxybenzoic acid.[6]

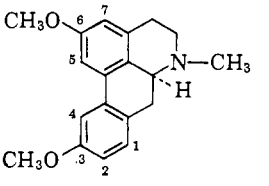
III was characterized as the methiodide,[7] $C_{20}H_{24}O_2NI$, m.p. 164–166°, $[\alpha]^{25}D$ +66° (methanol), and the Hof-



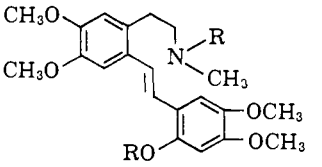
I



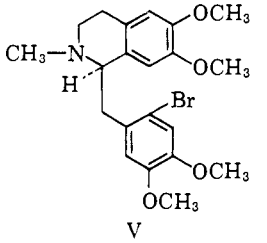
II



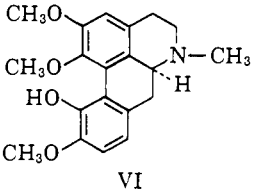
III



IVa, R = CH_3
b, R = CH_3CH_2



V



VI

(1) This is part II of a series entitled "Thalictrum Alkaloids"; part I is ref. 2.

(2) S. M. Kupchan, K. K. Chakravarti and N. Yokoyama, *J. Pharm. Sci.*, in press.

(3) Rotations and infrared spectra are in chloroform unless otherwise noted. All ultraviolet spectra are in methanol.

(4) N.m.r. spectra were determined on a Varian Associates recording spectrometer (A-60) at 60 Mc. in deuterated chloroform. Chemical shifts are reported in τ values (p.p.m.) [G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958)].

(5) We thank Professor K. Biemann and Dr. B. C. Das, Massachusetts Institute of Technology, for the mass spectral data.

(6) J. B. D. Mackenzie and A. Robertson, *J. Chem. Soc.*, 497 (1949). We thank Professor W. B. Whalley, School of Pharmacy, University of London, for an authentic sample of 2-ethoxy-4,5-dimethoxybenzoic acid.

(7) T. Kitamura, *J. Pharm. Soc. Japan*, **80**, 1104 (1960).

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).

(16) H. Corrodi and E. Hardegger, *Helv. Chim. Acta*, **38**, 2038 (1955).

(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.

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RECEIVED MARCH 15, 1963

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]

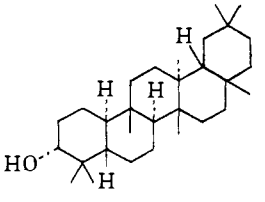
This alcohol, for which we propose the name tetrahymanol, was obtained as a white crystalline solid with m.p.[3] 312.5–314.5° (*Anal.*[4] Calcd. for $C_{30}H_{52}O$: C, 84.04; H, 12.23. Found: C, 84.04, 83.89; H, 12.09, 12.20)[5] by saponification of the material extracted from the lyophilized organisms[6] 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.