a solution of 0.13 g. of dihydro-γ-fagarine in excess methyl iodide to stand at room temperature for two days. A solution of 0.15 g. of the crude methiodide in 10 ml. of methanol was treated with 5 ml. of 10% sodium hydroxide solution and warmed on a steam-bath for 10 min. After evaporation of most of the methanol, the product was extracted with ether. The ether extracts were washed with 0.1 *N* sulfuric acid solution and sodium bicarbonate solution. The product crystallized from aqueous methanol as colorless needles, m.p. 120–121°. This material was identical (mixed m.p. and infrared spectrum) with that obtained from hydroxylunacridine.

Anal. Calcd. for $C_{14}H_{17}O_4N$: C, 63.86; H, 6.51; N, 5.32. Found: C, 63.89; H, 6.47; N, 5.23.

4,8-Dimethoxy-1-methyl-3-carboxaldehydoethyl-2-quinolone 2,4-Dinitrophenylhydrazone.—A mixture of 4,8-dimethoxy-1-methyl-3-(β-hydroxyethyl)-2-quinolone (70 mg.) from γ-fagarine, chromic acid (50 mg.) and pyridine (7 ml.) was allowed to stand at room temperature. The product was isolated by ether extraction of the reaction mixture after dilution with water. Treatment of the crude product with a solution of 2,4-dinitrophenylhydrazine in ethanol–sulfuric acid solution gave a yellow crystalline derivative, m.p. 213–215°, after crystallization from ethyl acetate. This was identical (mixed m.p., infrared spectrum) with the product from hydroxylunacridine.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Isolation of Alkaloids from *Balfourodendron riedelianum*. The Structure of Balfourodine

BY HENRY RAPOPORT AND KENNETH G. HOLDEN[1]

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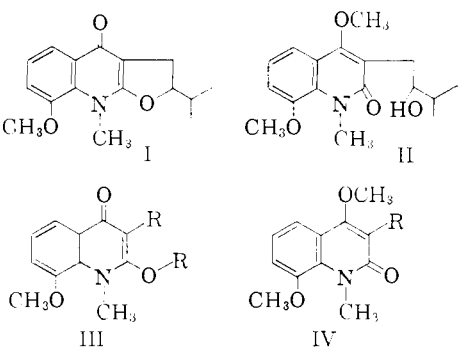
The alkaloids of *Balfourodendron riedelianum* were separated from the crude plant extract by a systematic extraction procedure which fractionated the alkaloids according to their basicities and polarities. The structures of two of the alkaloids, balfourodine and balfourolone, have been determined, and it has further been shown that balfourolone is an artifact of the isolation procedure arising from the base-catalyzed ring opening of an O^4-methylbalfourodinium salt whose structure is also discussed.

Balfourodendron riedelianum, a member of the Rutaceae family, is a small tree or shrub indigenous to Brazil and Argentina where it has found popular use for the treatment of stomach and intestinal ailments. Mundt[2] has reported the isolation of a small amount of alkaloidal material from the mature bark. In the present work the isolation of alkaloids was undertaken in a systematic manner and the structures of the alkaloids present in greatest concentration were determined.

Separation of the alkaloids from other plant material was accomplished by continuous extraction of an aqueous solution of the bark extract[3] with ether and more polar solvents at various *p*H's. By this method a fractionation of the alkaloids according to basicity and polarity was effected as shown in Fig. 1. The crude fractions thus obtained then were separated into fairly pure alkaloidal fractions by chromatography on alumina. Of immediate interest were the chief fractions from B and C, B_1 and C_1, since they, besides being the major alkaloidal fractions, had ultraviolet spectra which were suggestive of 2- and 4-quinolones,[4] respectively. Further investigation showed that the ultraviolet spectra of B_1 and C_1 were qualitatively identical with those of lunacrine (I) and lunacridine (II),[5] respectively. This suggests the part structures III for B_1 and IV for C_1. In view of these considerations and the ready availability of B_1 and C_1, a more detailed examination of these fractions was undertaken.



Compound B_1 upon further purification gave balfourodine, $C_{16}H_{19}O_4N$, m.p. 188–189°. Like lunacrine,[5] balfourodine contains one methoxyl and one N-methyl group, but differs in having an additional oxygen atom. The same relationship holds between lunacridine[5] and balfourolone, $C_{17}H_{23}O_5N$, m.p. 99–100°, obtained by crystallization from fraction C_1. Since the additional oxygen atom, in both compounds, has no effect on the ultraviolet absorption, it most certainly could not be on the quinolone nucleus. Therefore, a glycol grouping in balfourolone was strongly indicated.

Treatment of balfourolone with periodic acid followed by distillation of part of the reaction mixture gave acetone as its *p*-nitrophenylhydrazone. This establishes the part structure $R_2C(OH)C(OH)(CH_3)_2$ for balfourolone. The other fragment of the periodate oxidation was an aldehyde, $C_{14}H_{15}O_4N$, which was characterized as its semi-

(1) Public Health Service Predoctoral Research Fellow of the National Heart Institute.

(2) G. A. Mundt, *Anales farm. y bioquim. (Buenos Aires)*, [2] **21**, 25 (1954).

(3) We are indebted to Dr. Glenn E. Ullyot of Smith, Kline and French Laboratories, Philadelphia, and Dr. Oscar Ribeiro of Instituto de Quimica Agricola, Rio de Janeiro, for their assistance in procuring this material.

(4) E. A. Steck, G. W. Ewing and F. C. Nachod, THIS JOURNAL, **71**, 238 (1949).

(5) S. Goodwin and E. C. Horning, *ibid.*, **81**, 1908 (1959). We are indebted to Dr. Sidney Goodwin of the National Heart Institute for samples of lunacrine and lunacridine as well as for pre-publication information concerning her investigation of the alkaloids of *Lunasia amara* Blanco.

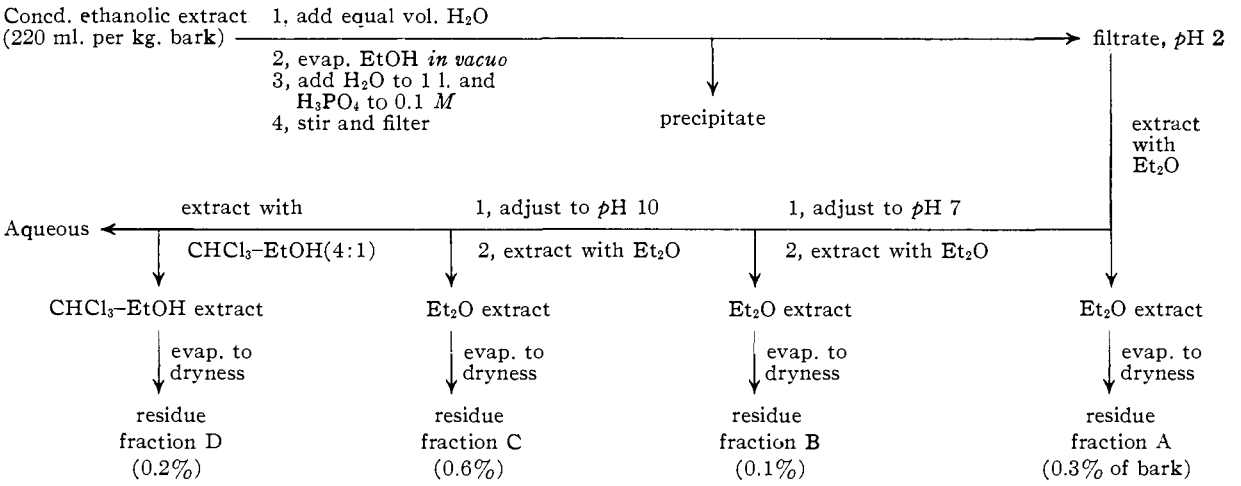
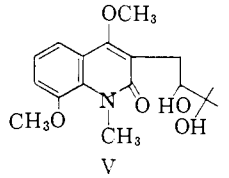
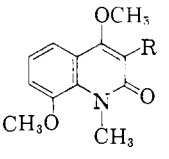


Fig. 1.—Flow sheet of separation of alkaloids of *Balfourodendron riedelianum* into main fractions.

carbazone. Reduction of this aldehyde with sodium borohydride gave a crystalline alcohol, $C_{14}H_{17}O_4N$, m.p. 116.5–118°. The analogy with lunacridine (II)[5] as well as the formation of acetone and the $C_{14}H_{15}O_4N$ aldehyde by cleavage with periodate led to the postulate of V as the structure of balfourolone. If this were true the cleavage aldehyde would have structure VI and the alcohol obtained from the reduction of this aldehyde would possess structure VII.

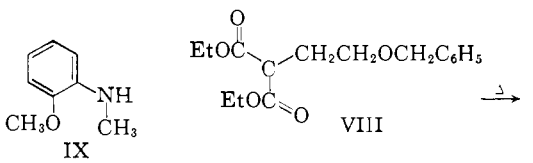


V

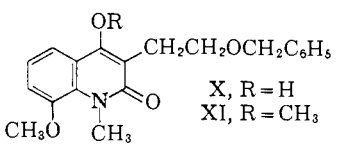


VI, R = CH_2CHO
VII, R = CH_2CH_2OH

In order to test this hypothesis the synthesis of the alcohol VII was undertaken. A general method for the synthesis of substituted 4-hydroxy-2-quinolones[6] consisting of the condensation of a substituted aniline with the appropriately substituted diethyl malonate was followed. Since the hydroxyl group required in the side chain of the alcohol VII must be protected in some way during the high-temperature ring closure, the benzyl ether of diethyl β-hydroxyethylmalonate (VIII) was prepared. Ring closure of this compound with N-methyl-*o*-anisidine (IX) in refluxing diphenyl ether gave the expected 1-methyl-3-(β-benzyloxy) ethyl-4-hydroxy-8-methoxy-2-quinolone (X). On treatment of X with diazomethane, 1-methyl-3-(β-benzyloxy)ethyl-4,8-dimethoxy-2-quinolone (XI) was obtained. Under mild conditions of hydrogenolysis this compound gave the desired alcohol VII in good yield. Comparison in the ultraviolet, infrared and by mixed melting point, showed the synthetic alcohol to be identical with that obtained from balfourolone. The structure of the aldehyde obtained from the periodate oxidation of balfourolone must then be VI and balfourolone is V.



IX VIII



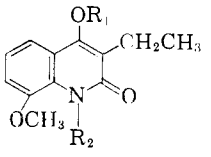
X, R = H
XI, R = CH_3

In connection with the proof of the structure of balfourolone it was at first though that the aldehyde VI could be reduced to the corresponding 1-methyl-3-ethyl-4,8-dimethoxy-2-quinolone (XII) by standard methods; therefore this compound was synthesized while the reduction of the aldehyde was being investigated. Following a procedure[7] for the preparation of 3-ethyl-4-hydroxy-7-methoxy-2-quinolone which called for heating the reactants together on a steam-bath for four hours prior to the high temperature ring closure, only XVI was obtained. However, when the reactants (*o*-anisidine and diethyl ethylmalonate) were added directly to refluxing diphenyl ether,[6] the desired 3-ethyl-4-hydroxy-8-methoxy-2-quinolone (XIII) resulted. Treatment of XIII with dimethyl sulfate in refluxing 20% aqueous sodium hydroxide gave a mixture of 1-methyl-3-ethyl-4,8-dimethoxy-2-quinolone (XII) and 3-ethyl-4,8-dimethoxy-2-quinolone (XIV) which were separated by chromatography. The necessity for this separation could be avoided by using diethyl ethylmalonate and N-methyl-*o*-anisidine to obtain 1-methyl-3-ethyl-4-hydroxy-8-methoxy-2-quinolone (XV) directly.

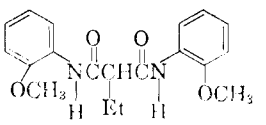
Although the desired ethyl compound XII had been synthesized, it had become evident that the reduction of the cleavage aldehyde VI to this compound could not be accomplished by the Wolff–Kishner reduction, by desulfurization *via* the ethylenedithioacetal, or by hydrogenolysis of the tosylate of the alcohol VII. In the latter instance treatment of the alcohol with *p*-toluenesulfonyl chloride in pyridine gave only a compound having

(6) M. F. Grundon, N. J. McCorkindale and M. N. Rodger, *J. Chem. Soc.*, 4284 (1955).

(7) R. G. Cook and H. F. Haynes, *Austral. J. Chem.*, **7**, 273 (1954).

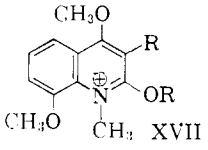


	R_1	R_2
XII	CH_3	CH_3
XIII	H	H
XIV	CH_3	H
XV	H	CH_3



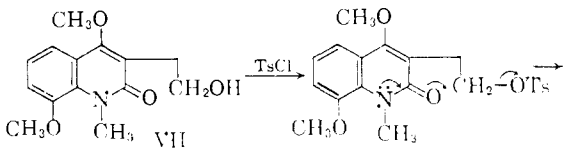
XVI

an ultraviolet spectrum requiring the part structure XVII. By analogy with the reaction of luna-

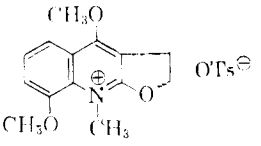


XVII

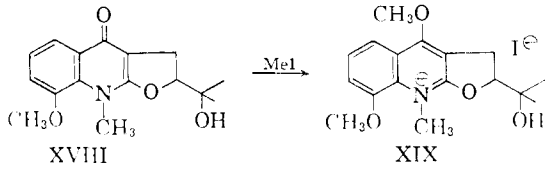
cridine[5] and α-toluenesulfonyl chloride, the following reaction is thought to occur



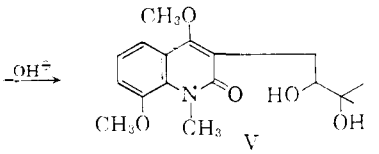
VII



With the structure of balfourolone established, balfourodine was examined in the light of the lunacrine → lunacridine[5] transformation and the very close similarity between these pairs of compounds. The methiodide of balfourodine was prepared by letting a solution of balfourodine in methyl iodide stand for a few days, following the procedure used for the preparation of lunacrine methiodide.[5] The precipitated methiodide had an ultraviolet spectrum qualitatively identical with that reported[5] for lunacrine methiodide. Under mildly basic conditions (pH 10), following the course of the reaction by the change in the ultraviolet absorption, it was determined that balfourodine methiodide was completely converted to balfourolone (V) in eight hours. The transformations observed can then be represented as



XVIII XIX



V

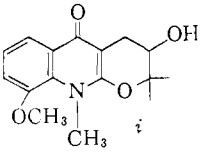
and structure XVIII[8] is assigned to balfourodine while its methiodide is O[4]-methylbalfourodinium iodide (XIX).

At this point, two observations aroused suspicion that balfourolone (V) might be an artifact of the isolation procedure. First, balfourolone was shown to have a distribution coefficient between ether and water which is largely in favor of ether and independent of the pH of the aqueous phase. This is significant since during the isolation procedure (see Fig. 1) the aqueous phase was extracted with ether at pH 2 and 7, but no balfourolone was found in the ether extracts; however, at pH 10 balfourolone was the only compound isolated from the ether extract (*ca.* 6 g. per kg. of plant). This suggests that balfourolone exists in the plant in some polar form which is very base sensitive, giving balfourolone in a relatively short time under mildly basic conditions (pH 10). Second, as shown above, the polar O[4]-methylbalfourodinium iodide (XIX) gives balfourolone almost quantitatively within eight hours.[10]

The presence of an O[4]-methylbalfourodinium salt in *Balfourodendron riedelianum* in sufficient quantity to account for all the balfourolone isolated was shown by varying the isolation procedure. After extraction with ether at pH 7 the aqueous phase was made 4 M in chloride ion by addition of sodium chloride. This step was necessary to ensure that the quaternary salt now would be extractable, as its chloride, since we have observed that the anion of a quaternary salt is very influential in determining its distribution coefficient between water and organic solvents. Thus it has been found that quaternary phosphates remain almost exclusively in the aqueous phase while halides are often extracted by organic solvents such as chloroform and butanol. For this reason phosphate has been used as the buffer during the isolation procedure to ensure that the alkaloids are extracted only as the free bases. In this case, however, it was desirable to extract a quaternary salt and hence chloride was added as the anion.

Extraction of the aqueous solution with butanol gave, on evaporation of the solvent, a residue which had an ultraviolet spectrum characteristic of a compound having the partial structure XVII. When the residue was taken up in absolute ethanol and perchloric acid was added, O[4]-methylbalfourodinium perchlorate precipitated. This, under

(8) The dihydrofurano structure for balfourodine and O[4]-methylbalfourodinium quaternary salt is much more likely than a pyrano structure, by analogy with lunacrine[5] and the other furoquinoline alkaloids.[9] However, the pyrano structure i cannot be ruled out on the available information since this could also give balfourolone by the above sequence.



(9) J. R. Price in "Progress in the Chemistry of Organic Natural Products," Vol. XIII, Springer Verlag, Vienna, 1956, pp. 317–329.

(10) During the isolation procedure the aqueous phase is adjusted to pH 10 and extracted continuously with ether for three days. Thus the eight hours required for the complete conversion of O[4]-methylbalfourodinium iodide to balfourolone is consistent with the extraction data.

mildly basic conditions (*p*H 10), gave balfourolone in good yield. From the ultraviolet spectrum of the purified perchlorate and that of the butanol extract from which the perchlorate was obtained, it was calculated that 27 mmoles of the O⁴-methylbalfourodinium salt was present in each kg. of plant. This corresponds to 8.6 g. of balfourolone per kg. of plant as compared to 6 g. per kg. of plant actually isolated by extraction at *p*H 10.

In order to establish beyond question that there was no additional precursor for balfourolone, such as a water-soluble ester which had not been extracted by butanol,[11] the aqueous phase was adjusted to *p*H 11 and allowed to stand for three days, thus converting any residual precursor (O⁴-methylbalfourodinium salt or other) to balfourolone. Extraction with ether gave 0.14 mmole of balfourolone per kg. of plant. Thus 99.5% of the precursor was extracted by butanol as the O⁴-methylbalfourodinium salt and this is the sole precursor of balfourolone.[12]

While the alkaloids of *Balfourodendron riedelianum* are similar in many respects to those of the *Lunasia* genus and, in fact, in one case appeared identical (balfourolone and hydroxylunacridine[13]) there is one very interesting difference. A comparison of the optical rotations of the alkaloids of the two genera shows that the structurally corresponding alkaloids of the two plants almost certainly have opposite absolute configurations (Table I). This

TABLE I

OPTICAL ROTATIONS[a] OF THE ALKALOIDS OF *B. riedelianum* AND *L. amara*

Compound	$[\alpha]^{25}D$	Compound	$[\alpha]^{24,25}D$
Balfourolone (V)	−36°	Hydroxylunacridine[13]	+31°
		Lunacridine (II)[5]	+30
Balfourodine (XVIII)	+49	Lunacrine (I)[5]	−50
O⁴-Methylhalfourodinium iodide (XIX)	+18	Lunacrine methiodide[5]	−26
O⁴-Methylbalfourodinium perchlorate (XIX perchlorate) from plant extract	+ 9	Methyllunacrinium picrate[b] from plant extract[12]	−20

[a] All rotations in ethanol. [b] Solvent and temperature not reported for methyllunacrinium picrate.

occurrence of enantiomorphous alkaloids in different species is quite uncommon and is reminiscent of the sinomenine alkaloids which are optically antipodal to the corresponding morphine derivatives. Other instances of this occurrence are the recent isolation of (+)-quebrachamine[14] and the first example of an enantiomorphous pair of flavonoids in nature.[15]

Experimental[16]

Isolation of Alkaloids from *Balfourodendron riedelianum*. A. Separation into Main Fractions.—To 650 ml. of concd. alcoholic plant extract (from 3 kg. of plant) was added one liter of water. The alcohol was removed from the mixture by evaporation under reduced pressure. After adding enough water and 85% phosphoric acid to make the final volume 3 liters and the concentration of phosphoric acid 0.1 molar, the mixture was stirred at room temperature for 2 hours to ensure complete solution of all basic material. Finally the mixture was filtered through filter-aid. The filtrate (*p*H 2) was extracted continuously with ether for 3 days; by adjusting the *p*H of the aqueous phase with sodium hydroxide similar extractions were carried out at *p*H 7 and 10. The aqueous phase was further extracted with chloroform–ethanol (4:1) for 9 days. The residues obtained by evaporation of the organic phases were designated as

Fraction	*p*H	Solvent	Wt., g.	%
A	2	Ether	8	0.3
B	7	Ether	3	.1
C	10	Ether	17	.6
D	10	$CHCl_3$–EtOH (4:1)	6	.2

B. Chromatography of Fractions.—Further purification of the various fractions obtained by extraction was achieved by chromatography of the fractions on alumina (Merck, 30 g. per g. of fraction). In each case, the material was applied to the column as a solution in chloroform, and the column was developed by successive elution with chloroform, chloroform–isopropyl alcohol, chloroform–methanol and finally with methanol. The various fractions were recombined according to their ultraviolet spectra. In this manner, four distinct substances were isolated from fraction A, whereas B, C and D each gave mostly a single alkaloidal fraction (B_1, C_1 and D_1).

C. Further Purification of Fraction B_1 and C_1. Balfourodine (XVIII).—Fraction B_1 was recrystallized four times from chloroform–benzene and sublimed at 170° (40 μ) to give balfourodine, m.p. 188–189°, $[\alpha]D$ +49°; infrared absorption: λ_{max} 6.17(s), 6.26(s), 6.42(s), 6.59(s), 6.69(s), 6.96(m), 6.72(m), 7.90(s), 9.31(s), 12.03(m) μ; ultraviolet absorption: λ_{max} 219 mμ (ϵ 22,000), 241 (43,500), 299 (10,600), 312 (11,100), 325 (9,600); in methanol 0.1 *M* in HCl: λ_{max} 214 mμ (ϵ 28,000), 257 (38,000), 299 (8,700), 315 sh. (4,300).

Anal. Calcd. for $C_{16}H_{19}O_4N$: C, 66.4; H, 6.6; N, 4.9; OCH_3, 10.7; NCH_3, 5.2. Found: C, 66.2; H, 6.6; N, 4.9; OCH_3, 10.5; NCH_3, 5.1.

Balfourolone (V).—Fraction C_1 was recrystallized twice from carbon tetrachloride–hexane to give balfourolone, m.p. 99–100°, $[\alpha]D$ −36°; infrared absorption: λ_{max} 6.15(s), 6.22(s), 6.31(s), 6.80(s), 6.92(m), 7.09(w), 7.31(s), 7.71(w), 8.05(s), 8.59(m), 8.89(m), 9.30(s), 10.11(m) μ; ultraviolet absorption: λ_{max}212 mμ (ϵ 24,000), 239 (25,000), 258 (27,000), 285 (8,200), 293 (7,800), 331 (3,500).

Anal. Calcd. for $C_{17}H_{23}O_5N$: C, 63.6; H, 7.2; N, 4.4; OCH_3, 19.3; NCH_3, 4.7; CCH_3, 4.7; mol. wt., 321. Found: C, 63.6; H, 7.3; N, 4.6; OCH_3, 19.8; NCH_3, 4.4; CCH_3, 5.6; mol. wt. (Rast), 307.

Periodate Oxidation of Balfourolone (V). A. Isolation of Acetone.—A methanolic solution of balfourolone (321 mg., 1 mmole, in 100 ml.) was treated with 100 ml. of 0.0379 *M* aqueous sodium periodate solution. At intervals 2-ml. aliquots were removed from the oxidizing solution and added to solutions prepared as follows: 2 ml. of sodium arsenite soln. (0.0411 equiv.), 10 ml. of water, potassium carbonate–sodium bicarbonate buffer, *ca.* 0.5 g. of potassium iodide. These solutions were titrated with standard periodate solution (0.001715 *M*) to a starch end-point. After three hours (100 mole % periodate consumed) the reaction had practically ceased and it was stopped by adding 10 ml. of 0.4 *M* arsenite solution (4 equiv.). The solution was distilled into an ice-cold aqueous solution of *p*-nitrophenylhydrazine hydrochloride until about 15 ml. of distillate had been collected and 65 mg. (34%) of a yellow precipitate was obtained from the oxidized solution of balfourolone. This precipitate was shown to be the *p*-nitrophenylhydrazone of

(11) The ultraviolet spectrum of the butanol extract showed that the O⁴-methylbalfourodinium salt chromophore was the only absorbing system present in detectable amount in the extract.

(12) A similar quaternary salt has been found in the *Lunasia* genus [J. R. Price in "Current Trends in Heterocyclic Chemistry," Academic Press, Inc., New York, N. Y., 1958, p. 92–99].

(13) S. Goodwin, J. N. Shoolery and E. C. Horning, THIS JOURNAL, **81**, 3736 (1959).

(14) F. Walls, O. Collera and A. Sandoval, *Tetrahydron*, **2**, 173 (1958).

(15) J. W. Clark-Lewis and D. G. Roux, *Chemistry & Industry*, 1475 (1958).

(16) All melting points are corrected and those above 200° were taken in evacuated capillaries; microanalyses were performed by the Microchemical Laboratory, University of California, Berkeley. Optical rotations were measured on 1% solutions in ethanol in one-decimeter tubes at 25°; infrared spectra were taken in chloroform and ultraviolet spectra were taken in methanol unless otherwise specified.

acetone by mixed melting point; m.p. 146–148° (reported[17] m.p. 149°), mixed m.p. 147–148°.

B. Aldehyde VI Fraction.—After the distillation to isolate the acetone, the methanol was removed from the reaction mixture at reduced pressure and the remaining aqueous solution was extracted continuously with ether for 12 hours. The ether phase gave 300 mg. of yellow oily material which was chromatographed on alumina (Merck, acid-washed) using hexane–benzene, benzene and benzene–methylene chloride for elution. The benzene-eluted fraction (200 mg.) was sublimed at 100° (50 μ) and gave partially crystalline, yellow-orange aldehyde; infrared absorption: λ_{max} 3.52(w), 3.64(w), 5.84(s) μ.

The semicarbazone of this aldehyde was prepared in the usual way and after recrystallizing twice from acetone and once from chloroform–benzene melted at 205.5–206°; ultraviolet absorption: λ_{max} 236 mμ (ϵ 35,000), 258 (33,000), 286 (9,800), 295 (8,800), 334 (3,800).

Anal. Calcd. for $C_{15}H_{18}N_4O$: C, 56.6; H, 5.7; N, 17.6. Found: C, 56.8; H, 5.7; N, 17.8.

Reduction of Aldehyde VI to Alcohol VII.—Aldehyde VI obtained directly from the periodate oxidation of 2.72 g. (8.45 mmoles) of balfourolone (V) was dissolved in 50 ml. of absolute ethanol and treated with 2 g. of sodium borohydride in 50 ml. of absolute ethanol. The resulting solution was stirred overnight at 0°. Excess sodium borohydride was then destroyed with 1 *N* HCl and the reaction mixture diluted to 200 ml. with water. After the ethanol had been removed under reduced pressure the remaining aqueous solution was extracted with chloroform (3 × 50 ml.). The organic phases gave 1.86 g. (7.04 mmoles, 83%) of alcohol VII which after two recrystallizations from acetone–hexane melted at 117–118°; infrared absorption: λ_{max} 6.12(s), 6.18(s), 6.28(s), 6.78(s), 7.32(m), 7.72(w), 8.07(m), 8.78(m), 9.00(m), 9.26(s), 9.59(m), 10.12(m) μ; ultraviolet absorption: λ_{max} 239 mμ (ϵ 25,000), 258 (26,000), 284 (7,600), 294 (7,300), 334 (3,200).

Anal. Calcd. for $C_{14}H_{17}O_4N$: C, 63.9; H, 6.5; N, 5.3. Found: C, 64.0; H, 6.5; N, 5.5.

1-Methyl-3-(β-benzyloxy)-ethyl-4-hydroxy-8-methoxy-2-quinolone (X).—Ethyl β-benzyloxyethylmalonate[18,19] (VIII) (10.7 g., 0.0366 mole) was prepared by treating benzyl chloroethyl ether[18,20] with the sodium salt of diethyl malonate. This malonic ester then was condensed with N-methyl-*o*-anisidine[21] (IX) (2.5 g., 0.0183 mole), prepared from N-formyl-*o*-anisidine[22] *via* lithium aluminum hydride reduction, by heating the two compounds together in refluxing diphenyl ether (15 ml.) for one hour while 1.25 ml. of ethanol was being evolved. After being cooled to room temperature, the reaction mixture was diluted with hexane (100 ml.) and the brown oil which separated was collected and chromatographed on 100 g. of alumina (Woelm, neutral, activity IV). The fractions were recombined on the basis of their ultraviolet spectra and the band eluted with benzene was sublimed at 150° (50 μ). Recrystallization from ether–hexane gave 1-methyl-3-(β-benzyloxy)-ethyl-4-hydroxy-8-methoxy-2-quinolone (X), m.p. 81–82°; ultraviolet absorption: λ_{max} 214 mμ (ϵ 30,000), 331sh. (30,000), 237 (31,000), 253 (28,000), 283 (8,400), 293 (8,600), 313sh. (4,900).

Anal. Calcd. for $C_{20}H_{21}NO_5$: C, 70.8; H, 6.2; N, 4.1. Found: C, 70.8; H, 6.1; N, 4.3.

1-Methyl-3-(β-benzyloxy)-ethyl-4,8-dimethoxy-2-quinolone (XI).—To an ethereal solution of 1-methyl-3-(β-benzyloxy)-ethyl-4-hydroxy-8-methoxy-2-quinolone (X) (0.61 g., 1.8 mmoles) was added a large excess of ethereal diazomethane. The reaction was carried out at 0°, the diazomethane being added over a period of 15 minutes. After standing overnight at room temperature the reaction mixture was shaken with 0.5 *M* phosphoric acid to destroy excess diazomethane. The ether phase gave 0.73 g. of an oil which was chromatographed on 20 g. of alumina (Woelm, neutral, activity IV). The fractions covering the solvent range hexane–benzene (9:1) to benzene were combined on the basis of their ultraviolet spectra. Molecular distillation of this material (150° (50 μ)) gave 1-methyl-3-(β-benzyloxy)-ethyl-4,8-dimethoxy-2-quinolone (XI) as an oil which was not purified further; ultraviolet absorption: λ_{max} 229 mμ (ϵ 25,000), 258 (25,000), 284 (9,000), 293 (8,200), 322sh. (3,400), 332 (3,600).

Anal. Calcd. for $C_{21}H_{23}NO_4$: C, 71.4; H, 6.5; OCH_3, 17.6. Found: C, 72.0; H, 7.0; OCH_3, 17.0.

1-Methyl-3-(β-hydroxy)-ethyl-4,8-dimethoxy-2-quinolone (VII).—An ethanolic solution of 1-methyl-3-(β-benzyloxy)-ethyl-4,8-dimethoxy-2-quinoline (XI) (136 mg., 0.386 mmole, in 15 ml.) was shaken with 29 mg. of 5% palladium-on-charcoal under hydrogen (1 atm.) for 4 hours. The reaction mixture was filtered and evaporated to dryness. Upon addition of ether, crystals separated which were recrystallized from acetone–hexane giving 1-methyl-3-(β-hydroxy)-ethyl-4,8-dimethoxy-2 quinolone (VII), m.p. 119°. This alcohol and the sodium borohydride reduction product (m.p. 117–118°) of the cleavage aldehyde showed identical ultraviolet and infrared absorption, and a mixture of the two melted at 118–119°.

3-Ethyl-4-hydroxy-8-methoxy-2-quinolone (XIII).—*o*-Anisidine (2.41 g., 19.6 mmoles) and diethyl ethylmalonate (5 g., 26.6 mmoles) were heated in refluxing diphenyl ether (8 ml.) for 1.5 hours under nitrogen. On cooling, the reaction mixture solidified. The solid was mixed with hexane (20 ml.) and filtered. After recrystallization from ethyl acetate and sublimation at 150° (10 μ), 3-ethyl-4-hydroxy-8-methoxy-2-quinolone (XIII) was obtained, m.p. 225–226°; ultraviolet absorption: λ_{max} 223 mμ (ϵ 19,000), 240 (26,000), 250 (22,000) 280sh. (6,700), 290 (7,900), 305 (6,000), 318sh. (4,800).

Anal. Calcd. for $C_{12}H_{13}O_3N$: C, 65.8; H, 5.9. Found: C, 65.7; H, 6.0.

When the above reactants, in equimolar amounts, were first heated together on a steam-bath for 4 hours prior to the ring-closure step, only the **diamide XVI** was isolated. After recrystallization from methanol–water and sublimation at 130° (20 μ), XVI melted at 153°; ultraviolet absorption: λ_{max} 248 mμ (ϵ 22,000), 283 (13,000), 290sh. (11,000).

Anal. Calcd. for $C_{19}H_{22}O_4N_2$: C, 66.7; H, 6.4; N, 8.2; OCH_3, 18.1. Found: C, 67.2; H, 6.3; N, 8.4; OCH_3, 17.8.

Methylation of 3-Ethyl-4-hydroxy-2-quinolone (XIII).—A solution of 2.5 g. (11.4 mmoles) of 3-ethyl-4-hydroxy-8-methoxy-2-quinolone (XIII) in 50 ml. of 20% aqueous sodium hydroxide was treated with 10 ml. of dimethyl sulfate. The mixture was boiled for 2 hours, 2-ml. portions of dimethyl sulfate being added at 15-minute intervals. The cooled reaction mixture was extracted with chloroform (3 × 50 ml.). The organic phases gave 2 g. of an oily material which was chromatographed on 60 g. of alumina (Merck). The first band eluted (methylene chloride) was an oil (1 g.) which was molecularly distilled at 80° (0.1 mm.) and was **1-methyl-3-ethyl-4,8-dimethoxy-2-quinolone (XII)**; ultraviolet absorption: λ_{max} 239 mμ (ϵ 26,000), 257 (22,000), 283 (7,100), 292sh. (6,200), 332 (3,100).

Anal. Calcd. for $C_{14}H_{17}NO_3$: C, 68.0; H, 6.9; N, 5.7; OCH_3, 24.6. Found: C, 67.7; H, 6.8; N, 5.6; OCH_3, 24.2.

A second band was eluted with methylene chloride–2% isopropyl alcohol and was crystallized from hexane to give **3-ethyl-4,8-dimethoxy-2-quinolone (XIV)**, m.p. 115.5–116°; ultraviolet absorption: λ_{max} 238 mμ (ϵ 28,000), 254 (43,000), 281 (12,000), 290 (7,800), 318sh. (3,100), 330 (3,900), 342sh. (2,500).

Anal. Calcd. for $C_{13}H_{15}NO_3$: C, 67.0; H, 6.4; OCH_3, 26.6. Found: C, 67.0; H, 6.5; OCH_3, 26.5.

1-Methyl-3-ethyl-4-hydroxy-8-methoxy-2-quinolone (XV).—A mixture of N-methyl-*o*-anisidine (5.15 g., 38 mmoles), 12 g. (64 mmoles) of diethyl ethylmalonate and 16 g. of diphenyl ether were heated under reflux for 3 hours (until evolution of ethanol ceased). Dilution of the cooled reaction mixture with hexane (100 ml.) gave a yellow precipitate which was recrystallized from benzene to give 3.14 g. (36%) of 1-methyl-3-ethyl-4-hydroxy-8-methoxy-2-quinolone (XV). This was further purified by recrystallizing it twice from benzene and subliming at 150° (20 μ), m.p. 188.5–189°;

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ultraviolet absorption: λ_{max} 217 mμ (ϵ 22,000), 239 (33,000), 254 (28,000), 282 (8,200), 293 (8,800), 315sh. (4,300).

Anal. Calcd. for $C_{18}H_{15}O_3N$: C, 67.0; H, 6.4; N, 6.0. Found: C, 67.2; H, 6.3; N, 6.3.

O^4-Methylbalfourodinium Iodide (XIX).—A solution of balfourodine (XVIII) (300 mg., 1.04 mmoles) in methyl iodide (25 ml.) was allowed to stand for 3 days. The white precipitate which formed was collected, and addition of hexane to a solution of this material in absolute ethanol gave O^4-methylbalfourodinium iodide which was extremely hygroscopic; λ_{max} 217 mμ (ϵ 43,000), 254 (36,000), 301 (8,100), 324sh. (4,600).

Anal. Calcd. for $C_{17}H_{22}NO_4I \cdot H_2O$: C, 45.5; H, 5.4; I, 28.2. Found: C, 45.9; H, 5.5; I, 27.7.

Balfourolone (V) from O^4-Methylbalfourodinium Iodide (XIX).—A solution of O^4-methylbalfourodinium iodide (200 mg., 0.46 mmole) in water buffered at *p*H 11 was allowed to stand for 16 hours. The solution was acidified to *p*H 1.5 and extracted with methylene chloride (3 × 25 ml.). Evaporation of the combined organic phases gave an oily substance which after recrystallization from ether gave balfourolone, m.p. 98–99°. A quantitative study using ultraviolet spectral data showed that at least 84% of the starting O^4-methylbalfourodinium iodide was converted to balfourolone within 8 hours and that there was no further change after this time.

Balfourolone Precursor; O^4-Methylbalfourodinium Perchlorate (XIX).—Starting with 327 ml. of plant extract (1.5 kg. of plant) the usual extraction scheme was followed through extraction at *p*H 7 with ether. At this point the aqueous phase was made 4 *M* in sodium chloride and extracted with butanol (3 × 800 ml.). On evaporation of the butanol at reduced pressure, 19.4 g. of solid material was obtained. This material was taken up in 200 ml. of absolute ethanol (about 2 g. remained undissolved). A 25-ml. portion of the ethanolic solution was treated with 15 ml. of 1 *N* perchloric acid in ethanol followed by dilution with 200 ml. of ether. A light yellow solid precipitated (about 1 g.) which after recrystallization from water and then from ethanol–ether melted at 124–125°, $[\alpha]_D$ +9°; ultraviolet absorption: λ_{max} 215 mμ (ϵ 31,000), 254 (36,000), 301 (7,500), 324sh. (3,700).

Anal. Calcd. for $C_{17}H_{22}NO_3Cl$: C, 50.6; H, 5.5. Found: C, 50.3; H, 5.8.

Conversion of O^4-Methylbalfourodinium Perchlorate (XIX) to Balfourolone (V).—O^4-Methylbalfourodinium perchlorate (0.5 g., 1.2 mmoles) was dissolved in water buffered at *p*H 10.5 and allowed to stand for 3 days. Upon extraction with methylene chloride (3 × 50 ml.), an oil (300 mg., 0.95 mmole) was obtained which on crystallization from ether gave balfourolone, m.p. 97–98°.

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[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE, THE WEIZMANN INSTITUTE OF SCIENCE]

Synthetic Studies on Sphingolipids.[1] III.[2] The Synthesis of Dihydrosphingomyelin[3]

BY DAVID SHAPIRO, H. M. FLOWERS AND SARAH SPECTOR-SHEFER

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The synthesis of benzoyl-, palmitoyl- and stearoyldihydrosphingomyelin (XXI) is described. *cis*-2-Phenyl-4-hydroxymethyl-5-pentadecyl-2-oxazoline (VII) is phosphorylated with β-chloroethylphosphoryl dichloride. The reaction product XVIb is hydrolyzed with diluted hydrochloric acid, and the resulting ester XIVb is acylated with the corresponding fatty acid chloride to give XIX. Quaternization with trimethylamine and removal of the benzoyl group by mild alkaline hydrolysis lead to the sphingomyelins XXIb,c. Conversion of XIVb to the barium salt XVIIa, followed by treatment with trimethylamine affords XXIa.

Sphingomyelin was discovered, in 1884, by Thudichum[4,5] who isolated it from an alcoholic extract of brain tissue. Subsequent workers later found that the main product of complete hydrolysis was the unsaturated base sphingosine (I), in addition to fatty acids, choline and phosphoric acid. The structural investigation of the sphingolipids which continued for about five decades until recent years was concentrated mainly on the chemistry of sphingosine.[6–10] Its structure was established by Carter and his collaborators in 1947.[11–12] Following this conclusion, the complete stereochemistry of sphingosine was soon determined by several investigators who were able to show that the carbons 2 and 3 have the *erythro* form,[13–18] and that the double bond has the *trans* configuration.[19–21] These results have been recently confirmed by synthesis.[2a,22,23]

The presence in sphingomyelin of phosphorylcholine as structural unit, an assumption which was based largely on analogy with the lecithins, has been recently substantiated.[24,25] The ester linkage of the phosphoric acid with the primary hydroxyl has been conclusively proved by Stotz and co-workers,[25,26] and the structure of sphingo-

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