tives appear to have a lower anesthetic efficiency (ratio of potency to toxicity) than the higher alkoxy derivatives. 2. Effect of varying the N-substituted group R′: (a) branched chain groups were less potent and toxic than straight chain groups; (b) heterocyclic and aromatic amino groups had a lower anesthetic efficiency than the alkyl amino groups.

Compounds 38, 40 and 41 proved to be more potent topically and less toxic than cocaine hydrochloride while compounds 13, 27, 29, 31, 32, 34, 35, 36, 40 and 41 were more potent on nerve block and infiltration anesthesia than procaine hydrochloride. The toxicity of this group ranged from equal to eight times as toxic as procaine hydrochloride. Several of these compounds when tried clinically in dental procedures were found to be as effective as procaine hydrochloride. The clinical evaluation is being continued and will be published elsewhere.

Experimental

The *o*- and *p*-anisole and phenetoles were obtained from commercial sources. The *p*-bromophenyl propyl ether and the *m*- and *p*-bromophenyl butyl and isoamyl ethers were prepared by alkylating the bromophenol with the appropriate alkyl halide, with a method similar to that described for alkylating nitrophenols.[6]

1-*p*-Ethoxyphenyl-3-chloropropanol-2.—The Grignard reagent prepared from 42 g. (1.8 moles) of magnesium and 300 g. (1.5 moles) of *p*-bromophenetole in 850 ml. of dry ether was treated with 280 g. (3 moles) of freshly distilled epichlorohydrin in one liter of dry ether with stirring. The mixture was refluxed for one hour after addition of the epichlorohydrin and allowed to stand overnight. To the mixture was added dropwise 150 ml. of water and then 850 ml. of a 20% sulfuric acid solution with stirring. The ether solution was separated, dried over anhydrous sodium sulfate and distilled over a water-bath to remove the ether. The residue was distilled under high vacuum and the fraction boiling 106–108° at 35 μ yielded 185 g. (58%) of a colorless liquid, $n^{20}D$ 1.5369, d^{20} 1.157, molecular refraction 57.94 (calcd. 57.43).

1-*p*-Ethoxyphenyl-3-*n*-propylaminopropanol-2 Hydrochloride.—A mixture of 25 g. (0.12 mole) of 1-*p*-ethoxyphenyl-2-chloropropanol-2, 40 g. (0.7 mole) of *n*-propylamine and 50 ml. of isopropyl alcohol was refluxed for 24 hours. The alcohol and excess amine were removed by evaporation on a steam-bath and the residue dissolved in 100 ml. of 2 *N* hydrochloric acid. The solution was extracted with three 50-ml. portions of ether to remove the unreacted chloropropanol and then made alkaline with 25 ml. of concentrated ammonium hydroxide solution, and extracted twice with 100-ml. portions of ether. The ether solution was dried over anhydrous sodium sulfate and the ether removed by distillation on a water-bath. The residue was distilled under high vacuum and the fraction at 125–127° at 30 μ was dissolved in 50 ml. of isopropyl alcohol and acidified with anhydrous hydrochloric acid to yield on recrystallization from isopropyl alcohol 19 g. (60%) of the anesthetic salt, m.p. 151–153°, as white crystals.

Acknowledgment.—We are indebted to Richard Sriubas for assistance in the analyses and to Michael Fisher for assistance in the pharmacological testing of these compounds.

BROOKLYN 7, N. Y.

(6) "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 140.

[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

Alkaloids of *Lunasia amara* Blanco. Isolation Studies

BY SIDNEY GOODWIN, A. F. SMITH, A. A. VELASQUEZ AND E. C. HORNING

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The isolation and characterization of fourteen leaf alkaloids of *Lunasia amara* Blanco are described.

A number of chemical and pharmacological studies of *Lunasia* alkaloids have been recorded since the initial observations of Lewin[1] and Boorsma,[1] but the structures of these compounds have been unknown until recently. Both leaves and bark of trees of this genus of the Rutaceae contain "water-soluble" alkaloids which are quaternary salts, and the organic bases include representatives of five classes of compounds. The following sections summarize what is known about the occurrence and structure of each of the alkaloids, and the Experimental section contains data relating to the isolation and characterization of the leaf bases of *Lunasia amara* Blanco of Philippine origin. With one exception (lunamaridine), all of the previously known bases have been found, together with a number of new ones. Table I contains formulas, melting points and yields of the leaf bases.

2-Arylquinolines.—An earlier report[2] described the isolation, structural identification and synthesis

(1) L. Lewin, "Lehrbuch der Toxikologie," 2nd Ed., Urban and Schwarzenberg, Vienna and Leipzig, 1897, p. 271; W. G. Boorsma, *Bull. Inst. Bot. Buitenzorg*, **6**, 15 (1900).

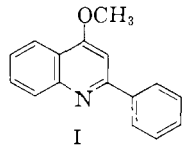
(2) S. Goodwin, A. F. Smith and E. C. Horning, THIS JOURNAL, **79**, 2239 (1957)

TABLE I

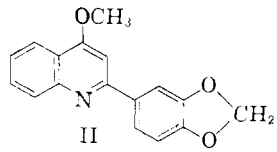
	Name	Formula	M.p., °C.	Yield,[a] %
I	4-Methoxy-2-phenylquinoline	$C_{16}H_{13}ON$	66–67	0.04
II	4-Methoxy-2-(3′,4′-methylenedioxyphenyl)-quinoline	$C_{17}H_{13}O_3N$	116–117	.002
III	7-Methoxy-1-methyl-2-phenyl-4-quinolone	$C_{17}H_{15}O_2N$	198–200	.002
IV	Lunamarine	$C_{18}H_{15}O_4N$	245–247	.004
V	Kokusagine	$C_{13}H_9O_4N$	195–197	.0003
VI	Skimmianine	$C_{14}H_{13}O_4N$	179–180	.0001
VII	Lunacrine	$C_{16}H_{19}O_3N$	117–118	.1
VIII	Lunine	$C_{16}H_{17}O_4N$	228–229	.004
IX	Hydroxylunacrine	$C_{16}H_{19}O_4N$	201–203	.002
X	Hydroxylunine	$C_{16}H_{17}O_5N$	228–230	.001
XII	Lunacridine	$C_{17}H_{23}O_4N$	85–86	...
XIII	Hydroxylunacridine	$C_{17}H_{23}O_5N$	100–102	.003
XIV	Hydroxylunidine	$C_{17}H_{21}O_6N$	124–125	.001
	Lunacrinol	$C_{16}H_{19}O_4N$	187–188	.0005
	Lunolone	.....	100–103	.001

[a] These yields are for pure material and are approximate.

of 4-methoxy-2-phenylquinoline (I). One of the new substances isolated during this study was found to have the formula $C_{17}H_{13}O_3N$, and the change in the ultraviolet absorption spectrum after acidification (a bathochromic shift) was similar to that observed for I. The behavior of the hydrochloride during a melting point determination



I

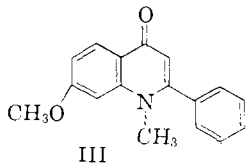


II

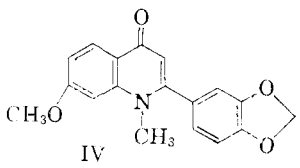
(formation of a quinolone) was also similar to that observed for I. Synthetic studies, to be summarized later, resulted in the identification of this substance as 4-methoxy-2-(3',4'-methylenedioxyphenyl)-quinoline (II).

Cusparine, galipine and other quinolines have been found in plants of the Rutaceae (*Cusparia sp.*), but this is the first occurrence of 2-arylquinolines noted for a natural source.

2-Aryl-4-quinolones.—The isolation, structural identification and synthesis of 7-methoxy-1-methyl-2-phenyl-4-quinolone (III) has been described by Johnstone, Price and Todd.[3] This quinolone was found in the bark of *L. quercifolia* (Warb.) Lauterb. and K. Schum collected in northern Australia. One of the leaf alkaloids of *L. amara* was found to have physical properties corresponding to this substance, and identity was established by direct comparison with an authentic sample provided by Dr. J. R. Price. Another leaf alkaloid, $C_{18}H_{15}O_4N$, m.p. 245–247°, seemed also from its properties to fall into this structural class. This compound is the **lunamarine** of earlier workers,[4] and synthetic studies (to be reported later) have established its structure as IV. It is of interest to note that the relationship between III and IV is the same as that found for I and II.



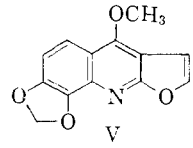
III



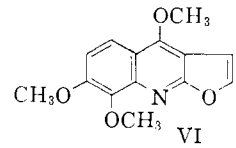
IV

Although these substituted 4-quinolones are a relatively recent addition to alkaloid structures, they may occur more often than their late discovery would indicate.[5] The quinolone III has also been found as a bark component of *Casimiroa edulis*,[6] a Mexican tree of the Rutaceae.

Furoquinolines.—Two optically inactive alkaloids, present in trace amounts, gave ultraviolet absorption spectra that resembled those of known naturally occurring furoquinolines. The analytical data and melting points, together with the absorption spectra, suggested that these compounds were kokusagine (V) and skimmianine (VI). The tentative identification in both cases was confirmed by comparison with authentic specimens.



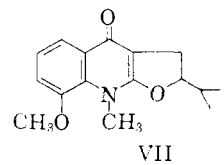
V



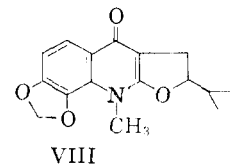
VI

The occurrence of these furoquinolines in a plant of the Rutaceae is not surprising. They were not detected in earlier studies, but their occurrence along with quinolines and quinolones in this instance suggests that some of the compounds of unknown structure remaining from *Orixa* work may be quinolines or quinolones. For example, orixine[7] ($C_{18}H_{21-23}O_6N$) may be a quinolone similar to lunacridine, and kokusagoline[7] ($C_{17}H_{13}O_5N$) may be a 2-aryl-4-quinolone. Both of these compounds occur in *Orixa japonica* along with skimmianine and kokusagine.

Dihydrofuro-4-quinolones.—The major alkaloid of *Lunasia sp.* is **lunacrine**. This compound has been found in every previous investigation[8] and it is now known to have the structure VII.[9,10] The assignment of position of the 8-methoxyl group is based on both ultraviolet spectra comparisons[9,11] and on the n.m.r. spectrum.[10] Another alkaloid, occurring in lower yield, corresponded to the **lunine** described by Boorsma.[12] This substance, m.p. 228–229°, was the same as a substance isolated[3] from *L. quercifolia*. The empirical formula was found to be $C_{16}H_{17}O_4N$. The analytical data indicated that one methylimino group was present but no methoxyl groups. The n.m.r. spectrum indicated that the ring system and the side chain structure were the same as that in lunacrine (VII), that a methylenedioxy group was present and that the aromatic substituents were placed as represented in VIII.[10] The long wave length maximum in the ultraviolet absorption spectrum



VII



VIII

of VIII was shifted bathochromically on acidification; this is contrary to the effect noted for lunacrine. The direction of the shift evidently depends on the nature and extent of alkoxyl substitution, and this effect has been confirmed in other work.

Two additional akaloids were found to be related to lunacrine and lunine. Both of these materials were present in small quantity, and it was

(3) E. R. Johnstone, J. R. Price and A. R. Todd, *Aust. J. Chem.*, **11**, 562 (1958); J. R. Price, "Progress in the Chemistry of Organic Natural Products," Ed. L. Zechmeister, Vol. 13, 1956, p. 302.

(4) F. A. Steldt and K. K. Chen, *J. Am. Pharm. Assoc., Sci. Ed.*, **32**, 107 (1943).

(5) Lunamaridine, $C_{16}H_{15}O_2N$, m.p. 209–210°, isolated by earlier workers[4] may well be a 2-phenyl-4-quinolone. Further work must await reisolation of the material.

(6) F. Sondheimer and A. Meisels, *J. Org. Chem.*, **23**, 762 (1958).

(7) M. Teraska, *J. Pharm. Soc. Japan*, **51**, 707 (1931).

(8) Summarized by Henry, "The Plant Alkaloids," Blakiston Co., Philadelphia, Pa., 1949, p. 751.

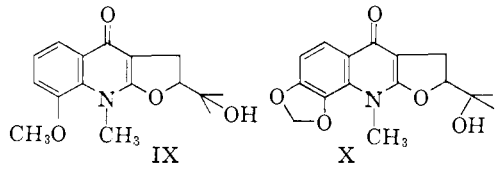
(9) S. Goodwin and E. C. Horning, THIS JOURNAL, **81**, 1908 (1959).

(10) S. Goodwin, J. N. Shoolery and L. F. Johnson, *ibid.*, **81**, 3065 (1959).

(11) J. R. Price, "Current Trends in Heterocyclic Chemistry," Academic Press, Inc., New York, N. Y., 1958, p. 92.

(12) W. G. Boorsma, *Bull. Inst. Bot. Buitenzorg*, **21**, 8 (1904).

difficult to obtain a satisfactory degree of analytical definition. One of them had an ultraviolet absorption spectrum that was identical with the very distinctive spectrum of lunacrine in both neutral (ethanol) and acid solution, and the infrared spectrum was identical in the 6 μ region with that found for lunacrine. Since the elementary analysis for the base perchlorate (perchlorate salts are generally the most satisfactory derivatives for 4-quinolones) indicated an additional oxygen atom over lunacrine, this compound was considered to be **hydroxylunacrine.** The n.m.r. spectrum indicated that the isopropyl-tertiary hydrogen atom was not present. Thus hydroxylunacrine was assigned structure IX.

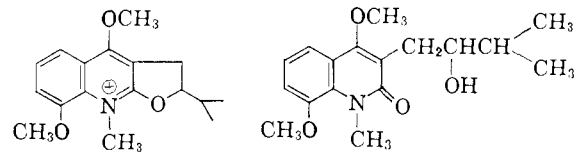


IX X

The tertiary alcohol structure assigned to the side chain is not unusual, and a similar situation was indicated by the n.m.r. spectrum for the alkaloid hydroxylunacridine (discussed later). For the latter compound, the chemical evidence was unequivocal and the side-chain structure was established as $-CH_2CHOHCOH(CH_3)_2$.[13] The position of the side-chain oxygen substitution was precisely that suggested for IX. Since the validity of the n.m.r. data was confirmed in this instance for hydroxylunacridine, there is no evident reason to doubt the applicability of the method to a closely related compound.[14]

The fourth dihydrofuroquinolone, $C_{16}H_{17}O_5N$, was found to have ultraviolet, infrared and n.m.r. spectra similar or identical to those found for lunine and, like lunine, the alkaloid was found to contain one N-methyl group but no methoxyl group. Since the relationships were the same as those found for hydroxylunacrine and lunacrine, this compound was evidently **hydroxylunine,** and the evidence suggested structure X. An additional alkaloid, **lunacrinol,** $C_{16}H_{19}O_4N$, isolated in very small amount, is described in the Experimental section. The 4-quinolone system is present according to the ultraviolet spectra measured in neutral (ethanol) and acidic solution.

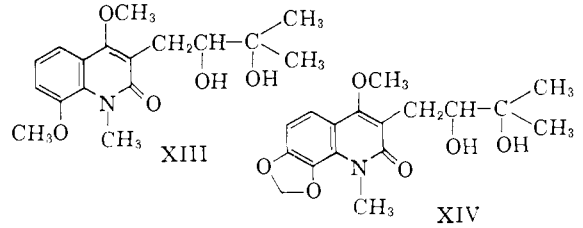
2-Quinolones.–The **lunacridine** of Boorsma[5,6,10,12] is now known to have structure XII.[9,11] This substance was not isolated in the course of this work, although it is formed from the methyl-



XI XII

lunacrinium ion XI under alkaline conditions. The "water-soluble" alkaloids, of which XI is the chief example, occur both in the bark and leaves of *Lunasia sp.*,[3,11] and it is possible that some or all of the 2-quinolones isolated in the present work and earlier are in fact derived from quinolinium salts present in the plant. The conventional isolation procedures generally involve sufficiently strong alkaline conditions to convert existing salts of the XI type to the corresponding quinolones.

Three 2-quinolones were recognized by their ultraviolet absorption spectra, and by the fact that these spectra were unchanged in acid solution (the weakly basic 2-quinolone structure forms salts only under very strongly acid conditions in suitable solvents). Two of these compounds were obtained in sufficient quantity for relatively complete study. One had the formula $C_{17}H_{23}O_5N$; the ultraviolet, infrared and n.m.r. spectra indicated that the aromatic part of the molecule was identical with that of lunacridine, and the n.m.r. data pointed to a side chain $-CH_2CHOHCOH(CH_3)_2$. This compound was assigned structure XIII and the name **hydroxylunacridine.** The structure has been confirmed through reaction relating the compound to dihydro-γ-fagarine.[13] The position of the side chain oxygen atoms, confirmed in this case by chemical degradation evi-



XIII XIV

dence, corresponds to that proposed for hydroxylunacrine and other compounds in this series. A similar situation was found for another optically active 2-quinolone with the formula $C_{17}H_{21}O_6N$. The ultraviolet absorption spectrum was different from that of XIII, but it was clearly that of 2-quinolone. The infrared spectrum supported this assignment. The presence of a methylenedioxy group was indicated by the n.m.r. spectrum and positive Labat test.[15] These data indicate that

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

(14) Structure IX has been proposed for balfourodine, an alkaloid of *Balfourodendron riedelianum*, by H. Rapoport and K. G. Holden, THIS JOURNAL, **81**, 3738 (1959). Balfourodine may be converted to balfourolone, a 2-quinolone whose structure is the same as that of hydroxylunacridine (XIII). A comparison of the optical rotation data for balfourolone and hydroxylunacridine, as given by Rapoport and Holden, indicates that the two 2-quinolones differ in configuration, and suggests that they are enantiomorphs. If this is correct, balfourodine and hydroxylunacrine should be enantiomorphs. Unfortunately, a definitive comparison of data relating to these alkaloids is not possible, since the *Lunasia* base was not obtained in analytically pure form, and the amount available was not sufficient for independent study of its conversion to (+)-hydroxylunacridine. The latter compound is in the (−)-lunacrine series.

(15) In the course of work with this series of compounds, it was frequently necessary to compare the validity of data relating to the presence or absence of a methylenedioxy group. No exceptions were found for the Labat test, but it is extremely sensitive and impure specimens containing only small amounts of methylenedioxy alkaloids will give positive results. The use of infrared absorption methods is quite reliable, as found by L. H. Briggs, L. D. Colebrook, H. M. Fales and W. C. Wildman (*Anal. Chem.*, **29**, 904 (1957)), but some of the bands may be obscured in compounds with other oxygen-containing rings, as, for example, the furoquinolines. In these instances n.m.r. spectra gave the best results; the methylenedioxy group was detected readily. In general, it was found advisable to rely on the infrared and the n.m.r. data, rather than on Labat test results.

this compound is hydroxylunidine (XIV), the methylenedioxy analog of hydroxylunacridine. Its relationship to lunine (VIII) and hydroxylunine (X) will be noted.

A third 2-quinolone, lunolone, was isolated in very small quantity, and is described briefly in the Experimental section. Its structure is unknown, although the 2-quinolone system is present according to the ultraviolet spectrum in neutral and acidic solutions.

Quinolinium Salts.—The "water-soluble" group of compounds was studied by Price,[3,11] and the major component was found to be the methyllunacrinium ion XI. These compounds were not investigated in this study. It is not certain whether the 2-quinolones of *Lunasia sp.* are derived entirely from quinolinium ions during the isolation procedure, or whether they exist independently in the plant. In either case the isolation of a 2-quinolone suggests the prior existence of a corresponding quinolinium salt.

Acknowledgments.—We are grateful to Dr. J. R. Price, C.S.I.R.O., Melbourne, for a stimulating exchange of information and for reference samples of III, VIII and XII. We are indebted to Dr. E. Ritchie, University of Sydney, for generous samples of skimmianine and maculine and to Prof. T. Ohta, Tokyo College of Pharmacy, who kindly supplied the sample of kokusagine. The assistance of the Section of Plant Introduction, Agricultural Research Service, U. S. Department of Agriculture, in obtaining and identifying the plant material and of Dr. Felipe R. Amos, Director, Bureau of Forestry, Department of Agriculture, Republic of the Philippines, in making available the collections are most gratefully acknowledged.

The interest and generosity of Dr. J. N. Shoolery and Mr. L. F. Johnson, Varian Associates, in providing the high resolution nuclear magnetic resonance spectra is greatly appreciated.

Experimental

Melting points were taken on a Kofler block. Rotations and ultraviolet spectra (sh = shoulder) were taken in absolute ethanol, except where noted. Infrared spectra were taken in chloroform, unless otherwise specified, and only a selected number of bands are given as characterization features of the substances (s = strong, m = medium and w = weak). The above instrumental measurements were made by Mrs. K. Warren, Miss P. A. Wagner, Miss C. Monaghan and Mr. F. L. Byers. Nuclear magnetic resonance spectra were determined in deuteriochloroform (Merck, Ltd.). Analyses were made by Mr. W. Manser, Zurich, Switzerland; Mr. J. F. Alicino, Metuchen, N. J., and Dr. W. Zimmerman, Melbourne, Australia. Plant extractions were carried out by Mr. D. L. Rogerson and Mr. J. Link.

Isolations.—The preparation of the crude alkaloid fraction for chromatography was described earlier.[2] This fraction which amounted to 0.7% of the dry weight of the leaves was subjected to chromatography on acid-washed alumina (Merck) in portions of 43 g. (chromatogram A) and 95 g. (chromatogram B); unless otherwise noted the elution of the alkaloid refers to chromatogram A in a continuation of the chromatogram mentioned in the first report.[2] The solvents used were reagent grade and were not purified further.

4-Methoxy-2-phenylquinoline (I).—The isolation of this alkaloid and a proof of structure have been reported.[2]

2-(3',4'-Methylenedioxyphenyl)-4-methoxyquinoline (II).—This alkaloid was eluted, in continuation of chromatogram A, with 9:1 benzene–ether. The product crystallized on standing and afforded a positive Labat test. The analytical sample was obtained from pentane–ethyl acetate as colorless prisms, m.p. 116–117°; infrared spectrum: 615m, 6.25s, 6.39w, 6.64s, 6.69s, 6.88s, 9.62s, 10.74m μ; ultraviolet spectra: λ_{max} sh224, 234, 274, 311 and sh323 mμ (log ϵ 4.55, 4.61, 4.28, 4.17 and 4.11, resp.), λ_{min} 258 and 291 mμ (log ϵ 4.13 and 4.13); 1 *N* hydrochloric acid: λ_{max} sh226, 245, sh280 and 351 mμ (log ϵ 4.33, 4.45, 3.99 and 4.24, resp.); λ_{min} 295 mμ (log ϵ 3.89).

Anal. Calcd. for $C_{17}H_{13}O_3N$: C, 73.11; H, 4.69; N, 5.02; OCH_3, 11.11. Found: C, 72.95; H, 5.29; N, 4.81; OCH_3, 7.30; $(N)CH_3$, 1.34; $(C)CH_3$, none.

Kokusagine (V).—Kokusagine was eluted with ether–ethyl acetate (1:1) and crystallized from ethyl acetate as optically inactive colorless needles, m.p. 192–194°. An analytical sample, m.p. 195–197°, was recrystallized from the same solvent; infrared spectrum: 6.06m, 6.13s, 6.33m, 6.53s, 9.36s, 9.56s, 10.25s, 10.86w μ.

Anal. Calcd. for $C_{13}H_9O_4N$: C, 64.20; H, 3.73; N, 5.76; OCH_3, 12.76. Found: C, 63.95; H, 3.66; N, 5.95; OCH_3, 12.75.

The isolated alkaloid was compared by mixed m.p. with maculine (m.p. 201–203°) and kokusagine (m.p. 199–201°); depression of m.p. was observed in the first case but not in the second. The infrared and ultraviolet spectra of the alkaloid isolated in this work and kokusagine were identical in all respects.

Skimmianine (VI).—Skimmianine was eluted immediately after kokusagine and was crystallized from ethyl acetate, m.p. 179–180°. The m.p. on admixture with an authentic specimen of skimmianine (m.p. 181–183°) was not depressed and the infrared spectra of the isolated material and the authentic sample were identical in all respects; infrared spectrum: 6.12s, 6.17s, 6.31s, 6.43w, 9.50s, 10.06s, 10.21m, 10.50m μ.

Anal. Calcd. for $C_{14}H_{13}O_4N$: C, 64.84; H, 5.05; N, 5.40; OCH_3, 35.91. Found: C, 65.12; H, 4.97; N, 5.51; OCH_3, 35.10.

Lunacrine (VII).—In the chromatogram under description, ethyl acetate, increasing amounts of chloroform in ethyl acetate and finally 3 l. of chloroform failed to elute any material. Then, rapidly, lunacrine (VII), 7-methoxy-1-methyl-2-phenyl-4-quinolone (III), lunine (VIII) and lunamarine (IV) were eluted with chloroform. Since the amount of lunacrine present was about ten times that of the other three alkaloids combined, purification of the minor constituents was tedious. Appropriate fractions were rechromatographed on aluminum oxide (Merck). Lunacrine, m.p. 117–119°, was eluted with ethyl acetate and crystallized from ethyl acetate–pentane. Details of characterization and structure have been reported.[9]

7-Methoxy-1-methyl-2-phenyl-4-quinolone (III) was eluted in later ethyl acetate fractions of a chromatogram which contained lunacrine and lunine. Lunacrine, due to its great solubility, was removed easily by crystallization of the fraction from benzene. The mixture of lunine (VIII) and III (0.351 g., m.p. 173–206°, from 0.962 g. total fraction) on crystallization from ethyl acetate afforded 0.097 g. of prismatic crystals, m.p. 223–226° (lunine). Concentration of the mother liquor afforded a mixture of prismatic crystals and thin rectangular plates. About 50 mg. of the plates were separated mechanically and crystallized from ethyl acetate, m.p. 198–200°. On direct comparison with an authentic specimen,[3] m.p. 199–200°, the m.p. was unchanged on mixture and the infrared and ultraviolet spectra were identical in all respects; infrared spectrum: 6.15s, 6.20s, 6.25s, 6.33m, 6.39m, 9.37m, 9.67w, 10.62w μ.

Lunine (VIII).—Fractions rich in lunine and free of lunacrine and III were combined and rechromatographed on aluminum oxide. Lunine was eluted with benzene–ethyl acetate (3:1) mixture. It crystallized from ethyl acetate as prisms, m.p. 227–230°, and from methanol–water as a mixture of prisms and needles, m.p. 224–226°. The analytical sample, m.p. 228–229°, was crystallized from ethyl acetate; $[\alpha]^{25}_{589}$ −38.5° and $[\alpha]^{25}_{436}$ −87.4° (*c* 0.926 in chloroform); infrared spectrum: 6.09s, 6.30s, 6.39vs, 6.59m, 9.42s, 9.56m, 10.67s μ; ultraviolet: λ_{max} 222, 247, sh268, 314 and 325 mμ (log ϵ 4.31, 4.60, 3.97, 4.02 and 4.01, resp.); λ_{min} 228, 280 and 319 mμ (log ϵ 4.28, 3.46 and 4.00, resp.); in 0.1 *N* hydrochloric acid: λ_{max} 257 and 332 mμ (log ϵ 4.61 and 3.86, resp.); λ_{min} 233 and 275 mμ (log ϵ 3.97 and 3.21, resp.).

Anal. Calcd. for $C_{16}H_{17}O_4N$: C, 66.88; H, 5.96; N, 4.88; $(N)CH_3$, 5.23. Found: C, 66.82; H, 6.04; N, 4.95; OCH_3, none; $(N)CH_3$, 5.74; $(C)CH_3$, 3.05.

Lunine Hydroperchlorate.—Lunine was converted to this salt in methanol–ether. The analytical sample, m.p. 232–236°, crystallized from the same solvent pair as long, colorless needles.

Anal. Calcd for $C_{16}H_{17}O_4N \cdot HClO_4$: C, 49.56; H, 4.68, N, 3.61. Found: C, 49.47; H, 4.72; N, 3.62.

Lunamarine (IV), the least soluble of the series, can be purified by crystallization from a variety of solvents. The analytical sample was crystallized from methylene chloride as colorless crystals, m.p. 245–247°, $[\alpha]^{25}_{589}$ −0.7° (*c* 0.410 g. in chloroform). Lunamarine gave a positive Labat test; infrared: 6.15s, 6.24s, 6.39m, 9.37m, 9.66m, 10.72w μ; ultraviolet spectrum: λ_{max} 228, 246, 250, 259, 317 and sh324 mμ (log ε 4.50, 4.48, 4.48, 4.51, 4.28 and 4.27, resp.); λ_{min} 239, 248, 255 and 287 mμ (log ε 4.46, 4.48, 4.44 and 3.83, resp.).

Anal. Calcd for $C_{18}H_{15}O_4N$: C, 69.89; H, 4.89; N, 4.53; OCH_3, 10.03; $(N)CH_3$, 4.86. Found: C, 69.79; H, 5.00; N, 4.52; OCH_3, 9.90; $(N)CH_3$, 4.74.

Lunolone, isolated only from chromatogram B, was eluted immediately after II with ethyl acetate–benzene (5:95). The crystalline solid which formed in the oily red residue was separated by trituration with ether–pentane. Lunolone crystallized from ethyl acetate–pentane as colorless prisms, m.p. 100–103°, $[\alpha]^{23}_{589}$ +20.6° and $[\alpha]^{23}_{436}$ + 54.7° (*c*, 0.996); infrared spectrum: 6.14s, 6.21m, 6.32 μ; ultraviolet spectrum: λ_{max} sh233, 240, 258, 287, and 296 and 322–333 mμ (log ε 4.34, 4.36, 4.37, 3.86, 3.86 and 3.60, resp.); λ_{min} 247, 278, 291 and 311 mμ (log ε 4.26, 3.80, 3.84 and 3.54, resp.); unchanged on acidification.

Hydroxylunacridine (XIII).—In the course of the development of chromatogram B, a yellow-brown color extended over the entire column; ethyl acetate (100%) was applied to the column, and the colored material was rapidly swept off. A single fraction (1.3 l.) was taken. The residue was a brown gum (20.08 g.) from which crystalline material could not be obtained. Neutral impurities were not present in any quantity, since 18.57 g. of material was recovered (as a red gum) after extraction of an ethyl acetate–ether solution of the residue with 2 *N* hydrochloric acid, followed by basification and extraction with chloroform. A portion of the gum was converted to a crystalline picrate; after crystallization from acetone this was shown to be identical with lunacrine picrate. Subsequent chromatography on aluminum oxide afforded lunacrine, III, XIII and XIV; the total 4-quinolones amounted to 41% and the total carbostyrils amounted to 54%. Attempts to verify the implication that carbostyril derivatives should not have been extracted with acid (while quinolinium derivatives would be), have not been carried out; further investigation is anticipated.

On chromatography of the gum, hydroxylunacridine was eluted with benzene–ethyl acetate (3:2); the product crystallized in the residue only after very long standing. The Labat test was negative (reddish color). Recrystallization from ethyl acetate–cyclohexane afforded colorless needles, m.p. 100–102°, $[\alpha]^{24}_{589}$ +31.5° and $[\alpha]^{24}_{436}$ +84.2° (*c* 0.990); infrared spectrum: λ_{max} 6.13s, 6.21m, 6.33s, 9.32s, 10.57w μ; ultraviolet spectrum: λ_{max} 239, 257, 284, 293, 332 and sh345 mμ (log ε 4.39, 4.39, 3.93, 3.90, 3.55 and 3.41, resp.); λ_{min} 224, 246, 275, 291 and 310 mμ (log ε 4.32, 4.32, 3.88, 3.898 and 3.37, resp.); no change on acidification.

Anal. Calcd. for $C_{17}H_{23}O_5N$: C, 63.53; H, 7.21; N, 4.36. Found: C, 63.67, 63.84; H, 6.84, 7.05; N, 4.29.

Hydroxylunidine (XIV) was eluted after XIII with benzene–ethyl acetate (2:3). The Labat test was positive. Crystallization from ethyl acetate–cyclohexane afforded rosettes of crystals, m.p. 124–125°, $[\alpha]^{23}_{589}$ +27.6° and $[\alpha]^{23}_{436}$ +74.3° (*c* 0.820); infrared spectrum: λ_{max} 6.09s, 6.16s, 6.27s, 9.41s, 9.54m, 10.64w μ; ultraviolet spectrum: λ_{max} 228, sh237, sh260, 267, 318 and sh330 mμ (4.43, 4.36, 4.33, 4.37, 3.94 and 3.83, resp.); λ_{min} 249 and 279 mμ (log ε 4.12 and 3.53, resp.); no change on acidification.

Anal. Calcd. for $C_{17}H_{21}O_6N$: C, 60.88; H, 6.31; N, 4.18; OCH_3, 9.25; $(N)CH_3$, 4.48. Found: C, 61.37, 61.39; H, 6.22, 6.45; N, 4.17; OCH_3, 9.68; $(N)CH_3$, 5.63.

Hydroxylunine X.—The fraction containing this alkaloid was eluted with 5% ethanol in chloroform. Trituration of the residue with benzene afforded a tan solid corresponding to about 30% of the fraction. The benzene solution was treated separately to yield lunacrinol and hydroxylunacrine. The solid material was purified by chromatography on silicic acid; the product was eluted with 5% methanol in chloroform. Crystallization from benzene gave a sample with m.p. 228–230°, $[\alpha]^{25}_{589}$ −5.9°, $[\alpha]^{25}_{436}$ −13.8° (*c* 0.276); infrared spectrum: 6.98m, 6.22w, 6.44vs, 6.91s, 9.39m, 9.52m, 10.64w μ; ultraviolet spectrum: λ_{max} 220, 246, sh268, 320 and 330 mμ; λ_{min} 224, 282 and 326 mμ. In 1 *N* hydrochloric acid: λ_{max} 223, 260 and 340 mμ; λ_{min} 233 and 277 mμ.

Anal. Calcd. for $C_{16}H_{17}O_5N$: C, 63.36; H, 5.65; N, 4.62; $(N)CH_3$, 4.96. Found: C, 63.48; H, 5.66; N, 4.33; $(N)CH_3$, 4.62; OCH_3, none.

Hydroxylunacrine (IX).—The benzene solution (from the preceding experiment) was carried through an acid extraction procedure to eliminate neutral material (a small quantity of brown tar resulted). The alkaloid fraction was placed in aqueous solution at *p*H 5, and the mixture was extracted with ether. The combined ether solutions were reserved for the isolation of lunacrinol. The aqueous solution was taken to *p*H 12 and extracted with ethyl acetate and with chloroform. The organic solutions (*p*H 12 extraction) yielded a solid that was recrystallized several times from ethyl acetate; m.p. 201–203°; this gave variable analytical data, and low and variable negative rotation values, but a satisfactory perchlorate was obtained. The infrared (6 μ region) and ultraviolet spectra were identical with those of lunacrine.

Hydroxylunacrine hydroperchlorate was recrystallized from methanol–ether to yield colorless rods, m.p. 216–218° dec., $[\alpha]^{24}_{589}$ −14.6°, $[\alpha]^{24}_{436}$, −23.2° (*c* 0.881); infrared spectrum: 2.81m, 6.03s, 6.20s, 6.50s, 6.69s μ (Nujol).

Anal. Calcd. for $C_{16}H_{19}O_4N \cdot HClO_4$: C, 49.30; H, 5.17; N, 3.59. Found: C, 48.99; H, 5.42; N, 3.62.

Lunacrinol.—The ether extract (preceding experiment) was examined; a new product was present which was purified by several recrystallizations from ethyl acetate. An analytical sample was obtained as colorless prisms, m.p. 187–188°, $[\alpha]^{24}_{589}$ −5.4°, $[\alpha]^{24}_{436}$ −11.0° (*c* 1.03); infrared spectrum: 6.11vw, 6.21m, 6.26m, 6.38ms, 6.43vs, 6.68s; ultraviolet spectrum: λ_{max} 243, sh300, 321, sh329–333 mμ; λ_{min} 275 mμ. In 1 *N* hydrochloric acid: λ_{max} 255, 305, sh325 mμ; λ_{min} 273 mμ. In 1 *N* potassium hydroxide (50% aqueous ethanol) after 24 hours: λ_{max} 250, 289, 300, sh320 mμ.

Anal. Calcd. for $C_{16}H_{19}O_4N$: C, 66.42; H, 6.62; O, 22.12; N, 4.84; OCH_3, 10.73; $(N)CH_3$, 5.20; H*, 0.35. Found: C, 66.29; H, 6.57; O, 22.7; N, 4.91; OCH_3, 9.36; $(N)CH_3$, 5.9; $(C)CH_3$, 4.6; H*, 0.52 (reaction at 95°).

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