Recrystallization produced 4.45 g. (68%) of 4-methoxyphenylethanediol, m. p. 82°. It is free of nitrogen.

Anal. Calcd. for $C_9H_{12}O_3$: C, 64.32; H, 7.17. Found: C. 64.50; H, 7.17.

Acknowledgment.—The authors are indebted to Mrs. Bruckner-Hatz and to Miss Kovács Oskolás for the microanalyses and to Prof. B. Issekutz, Jr., for the pharmacological assay of some compounds.

Summary

A new synthesis of hydroxyaryl N-alkylamino ethanols is described. Hydroxyaryl methyl ketones, respectively, their esters or ethers were oxidized by means of selenium dioxide with very good yields to the corresponding aryl glyoxals. Some hydroxyaryl, respectively, benzyloxyaryl glyoxals were reduced together with methyl amine, respectively, isopropylamine to the adrenalinelike aryl ethanolamines. Synephrine and N-isopropyl-nor-adrenaline were prepared with good yields. Phenylglyoxal and 4-methoxy phenylglyoxal gave under the same conditions only the corresponding glycols as in these cases condensation before reduction of the glyoxals did not take place.

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[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, NO. 1221, AND THE DEPARTMENT OF CHEMISTRY, OCCIDENTAL COLLEGE]

Alkaloids of *Dichroa febrifuga*. I. Isolation and Degradative Studies[1]

BY J. B. KOEPFLI, J. F. MEAD[2] AND JOHN A. BROCKMAN, JR.

A part of the war-born government sponsored research on malaria[3] was directed to a search for new antimalarials from plant sources. In papers by Liu[4] there is incidental mention of an antimalarial drug indigenous to Yunnan, and samples of this drug (SN 6355),[5] supplied by Liu, when tested in this country were found to be quite active against avian malaria. The samples were labeled "Chunine Leaf Powder" and further identified only as "from Saxifragaceae." The significance of the Saxifragaceae as a source of new antimalarials was further indicated when the Chinese drug Ch'ang Shan (SN 10767), reputed to be the roots of the Saxifrage, *Dichroa febrifuga*, Lour., also proved active in avian malaria. The availability of an adequate supply of Ch'ang Shan plant material led to this investigation the results of which have been briefly communicated by us.[6]

In 1943 when we began our work, the available information on Ch'ang Shan was meager and often contradictory.[7] Furthermore, we were particularly concerned as to the botanical identity of our plant material since the Ch'ang Shan supplied us had been obtained from Chinese herb stores either in this country or abroad. Comparison of some of our root material with herbarium specimens and information[8,9] later available indicate that the type of Ch'ang Shan used in this investigation is derived from the roots of *D. febrifuga*. Supporting evidence of a chemical nature was obtained when we were able to isolate from *D. febrifuga* (SN 6521), botanically identified at the time of collection in India, the same alkaloids isolated from our supply of Ch'ang Shan.

Since *D. febrifuga* has long been used in the treatment of "intermittent fevers" in southeastern Asia it is surprising that except for one reference to a possible glucoside, "dichroin(?),"[10] no chemical examination of the plant has been reported until lately. References to investigations which have appeared recently will be cited below.

Isolation and Characterization[11]

Isolation from Ethanol Extracts.—From crude basic fractions of *D. febrifuga* we have isolated two alkaloids for which we have proposed[6] the names febrifugine (DR 15381)[12] and isofebrifugine (SN 14821). Reports that this plant does not contain alkaloids[13,14] are probably explained by the fact that the alkaloidal content of the root material is small, 0.05 to 0.1%, and by the fact that Meyer's reagent which is commonly used as a test for alkaloids is remarkably insensitive for these bases.

(1) This work was done in part under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the California Institute of Technology, and in part by grants from the United States Public Health Service and the Research Corporation, New York.

(2) Present address: University of California at Los Angeles.

(3) "A Survey of Antimalarial Drugs, 1941–1945," ed. by F. Y. Wiselogle, J. W. Edwards, Ann Arbor, Michigan, 1946.

(4) S. K. Liu, Y. Chang, T. Ch'un and S. Tan, *Chinese Medical Jour.*, **59**, 575–577 (1941); and S. K. Liu, *National Medical Jour. of China*, **27**, 327 (1941).

(5) The survey number, designated SN, identifies a drug in the records of the Survey of Antimalarial Drugs, ref. 3.

(6) J. B. Koepfli, J. F. Mead and J. A. Brockman, Jr., THIS JOURNAL, **69**, 1837 (1947).

(7) K. Kimura, *China Journal*, **23**, 109 (1935).

(8) C. S. Jang, *Chinese Medical Jour.*, April–June, 1944.

(9) C. Crevost and A. Petelot, "Catalogue des Produits de l'Indochine," Vol. V, Part 1, Produits Medicinaux, p. 164 (1928); and K. Heyne, "De Nuttige Planten van Nederlandsch Indië," 2nd ed., Vol. I, p. 687; Buitenzorg (1927).

(10) C. Hartwich, Neue Arzneidrog, 1897, p. 125, C. Wehmer, "Die Pflanzenstoffe," Vol. I, 2nd ed., G. Fisher, Jena, 1929, p. 428.

(11) All melting points are corrected.

(12) The designation "DR" identifies a drug in the Antimalarial Drug Repository of the National Institute of Health, Bethesda, Maryland.

(13) I. M. Tonkin and T. S. Work, *Nature*, **156**, 630 (1945).

(14) D. Hooper, *Nature*, **157**, 106 (1946).

Crude Basic Fractions.—The ground root material was extracted exhaustively with hot 96% ethanol, and the extract evaporated to a thick paste. The paste was extracted with 0.1 *N* hydrochloric acid, the acid solution was extracted with chloroform, and the chloroform was discarded. The aqueous phase was then made basic with sodium carbonate and extracted with chloroform. Evaporation of the chloroform at room temperature left a crude basic residue, A.

The alkaline aqueous phase was next extracted with butanol, the butanol was extracted with *N* hydrochloric acid, and the acid solution was made basic and extracted with chloroform. Evaporation of the chloroform gave a crude basic residue, B.

Isofebrifugine.—A sample of the crude basic fraction, A, was triturated and washed with acetone until no more color was extracted. The light buff-colored solid remaining melted at 125 to 127°. This was crystallized from methanol or from chloroform–ethanol mixture by slow evaporation at room temperature. If prolonged heating is avoided, it will crystallize from hot methanol in colorless chunky prisms, m.p. 129–130°, $[\alpha]^{25}D$ +131° (*c* 0.35, chloroform). At room temperature it is approximately 0.5% soluble in water, 3 to 4% in methanol, 7% in chloroform, very soluble in methanol–chloroform mixtures, almost insoluble in acetone, and very insoluble in ether, benzene and petroleum ether. It is easily extracted from basic solution by chloroform (distribution coefficient, K = 10) and by butanol (K = 6).

Anal. Isofebrifugine. Calcd. for $C_{16}H_{19}O_3N_3$: C, 63.8; H, 6.4; N, 14.0. Found: C, 63.3, 63.5; H, 6.4, 6.0; N, 14.0, 14.1.

A comparison of the antimalarial activity of isofebrifugine with that of its mother liquors indicated that the latter still contained most of the activity. The properties of the compound responsible for this activity necessitated an extensive chromatographic study which ultimately led to the isolation of febrifugine and to the establishment of its homogeneity.

Several adsorbents were tried and found to be unsatisfactory, but new alumina gave a single, reproducible zone which yielded pure, crystalline febrifugine. When this chromatographic zone was divided into three equal parts and each section was eluted and worked up separately, three crops of crystalline material with identical properties were obtained. Such behavior is strongly indicative of a pure substance.

Some samples of reclaimed alumina, as well as samples of calcium carbonate, gave the curious "double zone" effect described by Schroeder[15] for silicic acid.

Febrifugine.—Pure febrifugine was isolated from the crude basic fraction, B, by chromatographing the material from chloroform solution on to ALORCO, Grade F, minus 80 mesh alumina, reground to pass 200 mesh, and mixed with one-half part of Celite. As a developer 2% methanol in chloroform was used. The columns were post-washed with chloroform so that the developer would not interfere with the streak. The zone of febrifugine was located by streaking the column with an aqueous solution containing 0.1% potassium permanganate and 1% sodium hydroxide. The zone was evidenced by an immediate green streak which soon bleached. Elution of the febrifugine was accomplished with methanol.

Under the proper conditions, isofebrifugine appears in the filtrate of the chromatogram and can be recovered and purified as indicated above.

To obtain pure febrifugine from the eluate it was evaporated to dryness at room temperature under vacuum. The residue was taken up in dilute hydrochloric acid, extraneous oil (extracted from the alumina) was removed by extracting with ligroin and chloroform, the solution was made basic and extracted with butanol, and the butanol extract was evaporated to dryness at room temperature under vacuum. The febrifugine left as a residue crystallized from ethanol in colorless needles, m.p. 139–140°, $[\alpha]^{25}D$ + 6° (*c* 0.5, chloroform) and + 28° (*c* 0.5, ethanol).

At room temperature febrifugine is approximately 1 to 3% soluble in water, ethanol, acetone and chloroform, very soluble in methanol–chloroform mixtures and in water–ethanol mixtures, and very insoluble in ether, benzene and petroleum ether. From basic solution it is easily extracted by butanol (K = 31) and by 1:4 butanol–chloroform mixture (K = 11) but not very well by chloroform (K = 0.7).

Anal. Febrifugine. Calcd. for $C_{16}H_{19}O_3N_3$: C, 63.8; H, 6.4; N, 14.0. Found: C, 63.5, 63.5; H, 6.1; 6.3; N, 13.9, 14.0.

Febrifugine dihydrochloride was prepared by adding a 10% excess of 12 *N* hydrochloric acid to febrifugine dissolved in sufficient absolute ethanol so that the final solvent was 90 to 95% ethanol. It was recrystallized from 90% ethanol, m.p. 220–222° (dec.).

Anal. Febrifugine dihydrochloride. Calcd. for $C_{16}H_{21}O_3N_3Cl_2$: C, 51.3; H, 5.7; N, 11.2; Cl, 18.9. Found: C, 51.2, 51.3; H, 5.6, 5.8; N, 11.2, 11.2; Cl (Carius) 18.6, (ionic) 18.9.

Dimorphism of Febrifugine.—A crude base melting at *ca.* 150° was occasionally encountered during our early isolation studies, and at one time was thought to be a third alkaloid, isomeric with febrifugine and isofebrifugine. Subsequently the base has been obtained from water by the addition of the theoretical amount of 2.5 *N* sodium hydroxide to a saturated solution of febrifugine dihydrochloride and from spontaneous crystallization of melted isofebrifugine. The highest melting point obtained for this base is 154–156°, $[\alpha]^{25}D$ + 28° (*c* 0.5, ethanol).

A comparison with febrifugine revealed that both bases, in addition to having the same optical rotation, give the same zone when chromatographed on alumina under standard conditions and give the same dihydrochloride. Furthermore, a chloroform solution of febrifugine at room temperature, seeded with a crystal of the high melting base, deposits crystals of that base on slow evaporation, and conversely a chloroform solution of the high melting base gives febrifugine when appropriately seeded. This would appear to be conclusive evidence that the base of m.p. 154–156° is not an isomer of febrifugine but merely a higher melting modification and that febrifugine is dimorphic.

Interconversion of Isofebrifugine and Febrifugine.—In the early stages of this investigation attempts to characterize these alkaloids were greatly complicated by apparent anomalies in physical behavior. These difficulties were resolved when febrifugine was found to be dimorphic (see above) and when the bases were found to be readily interconvertible.

A 51-mg. sample of isofebrifugine was refluxed for one-half hr. in 10 ml. of absolute ethanol. The solution was

(15) W. A. Schroeder, "Annals N. Y. Acad. Sci.," Vol. XLIX, Art. 2, p. 211 (1948).

evaporated to a small volume and seeded with febrifugine to give a yield of 13.3 mg. of febrifugine, m.p. 138–139°. The mother liquors after further refluxing with alcohol for two hours, yielded an additional 12.5 mg. of febrifugine, and, on addition of hydrochloric acid, 7.7 mg. of febrifugine dihydrochloride. Isofebrifugine (74 mg.) was heated for a few minutes at 135° until crystallization took place (in some cases seeding was necessary). The solid was washed with acetone and dried to give 53 mg. of the higher melting form (154–156°) of febrifugine.

A 52-mg. sample of febrifugine was refluxed in 25 ml. of chloroform for three hours. The solution was chromatographed on alumina. The yield of crude isofebrifugine was 22.3 mg., which on recrystallization gave 16.0 mg. of pure isofebrifugine, m.p. 129–130°.

Isolation from Acid Extracts.—With a few centigrams of the pure alkaloids available it was possible to determine some of their properties and devise a far more satisfactory extraction and isolation procedure.

Isolation of **Febrifugine and Isofebrifugine by Adsorption on Fuller's Earth.**—The plant material was ground to 8 to 10 mesh and extracted with portions of 0.1 *N* hydrochloric acid until it was essentially free of alkaloids as indicated by a test of the extract with Dragendorff's reagent. The concentration of alkaloids in the combined extracts was then at least 75 μg./ml. Dragendorff's reagent is a sensitive test for the alkaloids, three drops of the reagent giving an immediate cloudiness with 1 ml. of 0.1 *N* hydrochloric acid containing 12 μg. of alkaloids, a barely perceptible cloudiness in one-half to one minute with 6 μg., and a clear solution with 3 μg. The test is not critically dependent on the acid concentration, but the sensitivity is somewhat less, and the precipitate forms more slowly at higher concentrations of acid.

The combined extracts were stirred for a few hours with fuller's earth and sufficient 12 *N* hydrochloric acid to keep the mixture acidic to congo red. A crude adsorption isotherm indicated that 150 g. of fuller's earth under these conditions is sufficient to adsorb more than 99% of 1 g. of the alkaloids. An amount of Celite equal to one-third the weight of fuller's earth was added, and the mixture was filtered through a pad of Celite weighing about one-sixth the weight of fuller's earth.

The filter cake was mixed with enough sodium carbonate solution (6 g. per 100 ml.) to make a thin paste, three volumes of butanol were added, and the mixture was vigorously agitated for about two hours. The butanol layer was separated and a second and third extraction made.

The combined wet butanol extracts were diluted with one-half volume of ligroin (60–70°) and extracted with dilute hydrochloric acid. The acid extracts were made basic with sodium carbonate and extracted with 1:4 butanol–chloroform mixture, and the extraction into acid and back into butanol–chloroform mixture from basic solution was repeated. The butanol–chloroform solution was then dried with sodium sulfate and evaporated almost to dryness at reduced pressure and room temperature. A light-colored solid separated, and it was mixed with a little ether and filtered off. Evaporation of the filtrate gave a second crop.

The crude alkaloids were taken up in sufficient hot absolute ethanol so that after a 10% excess of 12 *N* hydrochloric acid was added to convert the material to dihydrochlorides the final solvent was 90 to 95% ethanol. The mixture was seeded with febrifugine dihydrochloride and allowed to crystallize overnight in the cold. The febrifugine dihydrochloride obtained can be recrystallized by dissolving it in a minimum of hot 1:1 water–ethanol mixture, and adding sufficient absolute ethanol to make the final concentration of ethanol 90%.

The mother liquors from the febrifugine dihydrochloride were taken almost to dryness at room temperature, the residue taken up in a small amount of water, sufficient sodium carbonate added to make the solution basic, and the mixture extracted with chloroform. Evaporation of the chloroform at room temperature gave crude isofebrifugine which was purified by recrystallization from hot methanol.

Mother liquors from the above crystallizations are best worked up for additional material by chromatographing them from chloroform on alumina as previously described.

Results with *D. febrifuga*.—The fuller's earth adsorption method has given higher and more consistent yields of the alkaloids than could be obtained from ethanol extracts. The results of applying the method to the various plant materials available to us indicated that the root material of Chinese origin had a crude base content of 0.08–0.1% and that 55% of this consisted of febrifugine and isofebrifugine. The ratio of febrifugine to isofebrifugine varied from 6:1 to 1:1 which is not surprising in view of their interconvertibility. The root material from India had a crude base content of 0.05% of which 63% was febrifugine and only 2% isofebrifugine. Although botanically identified leaf material from China was not available to us, that from India had a crude base content of only 0.01–0.02% of which 50% was febrifugine (the presence or absence of isofebrifugine was not confirmed).

Results with "Chunine Leaf Powder."—Liu[4] in mentioning the leaves of a plant called by him "Chunine tree" stated that the antimalarial activity was due to an alkaloid "chunine," but he gave no experimental evidence whatsoever for the presence of alkaloids.

Since our interest in antimalarials of Chinese origin was first occasioned by Liu's report, we made numerous unsuccessful attempts to learn something of the nature of his "chunine leaf powder" from the small supply available. In this connection the effectiveness of the fuller's earth adsorption method was demonstrated in that we have now been able to isolate from a 25-g. sample of Liu's material (SN 6355), 8 mg. of febrifugine, identified by melting point, dihydrochloride, and absorption spectrum. This supplies experimental evidence for the presence of an alkaloid in Liu's material and suggests that the material was derived in whole or in part from *D. febrifuga* or a related plant.

Pharmacology.[16]—Isofebrifugine when given intravenously three times a day to ducks infected with *P. lophurae* was found to have a *Q* of 1 (tests G-5 and I-2), whereas it was found to be inactive at the maximum tolerated dose in test D-1 when given intravenously two times a day.

Febrifugine has a *Q* of 100 when tested against *P. lophurae* in ducks (tests G-5 and I-2) and a *Q* of 64 against *P. gallinaceum* in chicks (test A-1). When tested against the trophozoites of *P. cynomolgi* in the monkey it is at least 100 times as active as quinine.[17]

Studies of the acute oral toxicity of febrifugine[17]

(16) Description of the avian tests, A-1, D-1, G-5 and I-1, together with a definition of *Q*, the quinine equivalent, will be found in ref. 3.

(17) Private communication from Dr. L. H. Schmidt, Christ Hospital, Cincinnati, Ohio.

indicate that the LD_{50} for the white mouse is *ca.* 2.5 to 3.0 mg. per kg. This is somewhat more than 100 times that of quinine but the toxic symptoms are quite different. Febrifugine is slower in its action, giving rise to symptoms some two to four hours after administration. These symptoms include effects on respiration, urinary incontinence, sweating and a corrosive effect on the gastric mucosa.

Experiments on subacute toxicity were carried out with rhesus monkeys.[17] An animal receiving 0.3 mg. per kg. daily (administered in three doses, one every eight hours) survived sixteen days of treatment with no untoward reactions. An animal receiving 0.75 mg. per kg. daily succumbed on the ninth day of treatment, and marked hyperemia of the gastric mucosa (but no frank ulceration) was the only finding of note on autopsy. Febrifugine did not affect the formed elements of peripheral blood or bone marrow and did not produce methemoglobinemia or cyanosis.

Preliminary results[18] of a clinical trial of febrifugine indicate that while the drug reduces the parasite count when given in four oral doses totaling 2.5 mg. per day it is at the same time such a powerful emetic as to probably preclude its clinical use in malaria.[19]

Structural Studies[11]

A potentiometric titration of isofebrifugine indicated two basic groups of pK_{B_1} 5.7 and pK_{B_2} 12. A comparative value of pK_{B_1} 6.3 for febrifugine was calculated from distribution coefficient values around *p*H 7.

Zeisel determinations for methoxyl groups were negative for both alkaloids. The Herzig and Meyer determination for N-methyl gave varying results of *ca.* 1% with either alkaloid whereas the theoretical value for one such group is 5%.

Both alkaloids reduce Tollens reagent at room temperature, but a number of color tests for aldehydes or methyl ketones (Schiff reagent, sodium nitroprusside, *o*-dianisidine in glacial acetic, and sodium bisulfite) were negative.

Acetylation and benzoylation of the alkaloids gave oily products which could not be crystallized.

TABLE I

MELTING POINTS

Compound	Febrifugine	Isofebrifugine
Free base	139–140°	129–130°
	154–156°	
Dihydrochloride	220–222° (dec.)	Hygroscopic
Dibenzenesulfonyl	148.0–148.5°	182.5–183.5°
Semicarbazone	187–188° (dec.)	.....
Oxime	224–225° (dec.)	.....
N-Nitroso	171.5–172.5°	185–186.5°
Dihydro	192–193°	156.5–157.5°

(18) Private communication from Dr. G. Robert Coatney, National Institute of Health, Bethesda, Maryland.

(19) For recent reports of the clinical use of extracts of Ch'ang Shan in the treatment of malaria see ref. 8 and C. F. Tsu, *J. Trop. Med. and Hyg.*, **50**, 75 (1947).

Several crystalline derivatives of the alkaloids have been prepared and analyzed, and their melting points and those of the free bases may be compared in Table I.

Benzenesulfonyl Derivatives.—The alkaloids were treated overnight with excess benzenesulfonyl chloride in pyridine. Addition of water and dilute acid caused the derivatives to precipitate. The derivative of isofebrifugine was recrystallized from chloroform–ethanol mixture and from aqueous ethanol, and that of febrifugine from aqueous ethanol. Both derivatives were insoluble in dilute sodium hydroxide and dilute hydrochloric acid.

Anal. Calcd. for $C_{28}H_{27}O_7S_2N_3$: C, 57.9; H, 4.7; N, 7.2. Found for isofebrifugine: C, 57.9; H, 4.6; N, 7.3. Found for febrifugine: C, 57.8; H, 5.0; N, 7.3.

Febrifugine Semicarbazone and Oxime.—Febrifugine dihydrochloride was treated overnight with sodium acetate and either semicarbazide hydrochloride or hydroxylamine hydrochloride in aqueous solution. The derivative separated when the solutions were made just basic with sodium hydroxide. The semicarbazone was recrystallized from ethyl acetate or absolute ethanol and the oxime from aqueous ethanol.

Anal. for semicarbazone: Calcd. for $C_{17}H_{23}O_3N_6$: C, 56.9; H, 6.4; N, 23.4. Found: C, 57.1; H, 6.5; N, 23.1.

Anal. for oxime: Calcd. for $C_{16}H_{20}O_3N_4$: C, 60.8; H, 6.3; N, 17.7. Found: C, 60.9; H, 6.2; N, 17.5.

Under similar conditions isofebrifugine did not give an oxime or semicarbazone.

Nitroso Derivatives.—The alkaloids were treated in dilute hydrochloric acid with excess sodium nitrite for one-half hour at 0°. The nitroso compounds separated when the solutions were made basic. N-Nitrosofebrifugine was recrystallized from ethanol and N-nitrosoisofebrifugine from ethanol and from butanone.

Anal. Calcd. for $C_{16}H_{18}O_4N_4$: C, 58.2; H, 5.5; N, 17.0. Found for febrifugine: C, 58.4; H, 5.7; N, 17.1. Found for isofebrifugine: C, 58.1; H, 5.7; N, 17.2.

Absorption Spectra.—The absorption spectra of febrifugine and isofebrifugine are almost identical. In Fig. 1 the ultraviolet absorption spectra for febrifugine, 2-methyl-4-quinazolone, and 3-allyl-4-quinazolone in ethanol are shown. The striking similarity of the spectrum of febrifugine and that of the 3-substituted-4-quinazolone is evident. The maxima of the spectra of febrifugine (and isofebrifugine) and 3-allyl-4-quinazolone show a bathochromic shift in acidic solution, but not in basic solution. The maxima of the spectrum of the 3-unsubstituted-4-quinazolone show a bathochromic shift in both acidic and basic solution. Molal extinctions calculated for the extremes of these various spectra, assuming a molecular weight of 301 for the alkaloids, likewise show a remarkable agreement, the difference in corresponding values being in general less than 10%.

Absorption spectra were taken in absolute ethanol which had been redistilled from all-glass apparatus. The spectra were taken at concentrations which gave extinctions between 0.3 and 0.9 as read with a Beckman quartz spectrophotometer, model DU.

2-Methyl-4-quinazolone was synthesized by the method of Niementowski,[20] m.p., 238–241°, and 3-allyl-4-quinazolone was synthesized by the method of Hanford, *et al.*,[21] m.p. 65–66°.

(20) J. Niementowski, *J. prakt. Chem.*, **51**, 564 (1895).

(21) W. E. Hanford, P. Liang and R. Adams, THIS JOURNAL, **56**, 2782 (1934).

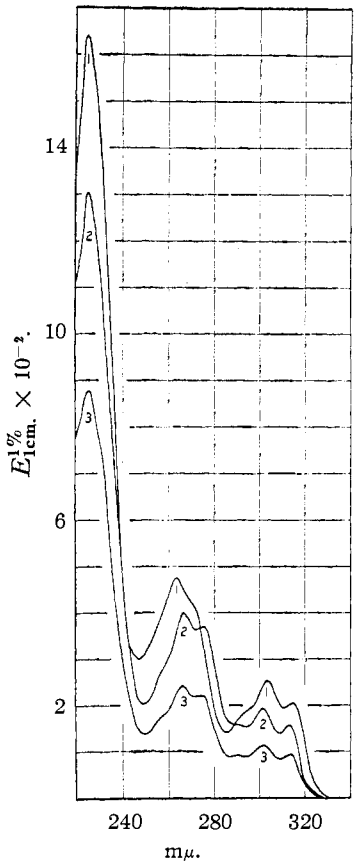

Fig. 1.—Absorption spectra in absolute ethanol: (1), 2-methyl-4-quinazolone; (2), 3-allyl-4-quinazolone; and (3), febrifugine.

Reduction.—In ethanol in the presence of platinum at room temperature and pressure both alkaloids take up one mole of hydrogen. Crystalline dihydro derivatives can be isolated, but in yields which suggest that each reduction leads to the formation of more than one product. In glacial acetic acid, febrifugine takes up one mole and isofebrifugine two or more moles of hydrogen, but in neither case could crystalline products be isolated.

Absorption spectra of the dihydro-alkaloids are very nearly the same as those of the original alkaloids. Dihydrofebrifugine does not form an oxime nor a semicarbazone. Neither of the dihydro-alkaloids reduces Tollens reagent, and both give negative results in N-methyl determinations.

Dihydrofebrifugine.—A 409-mg. sample of febrifugine was hydrogenated at room temperature and one atmosphere pressure in 50 ml. of absolute ethanol in the presence of 50 mg. of Adams catalyst. In one hour the volume of hydrogen taken up became constant and corresponded to one mole of hydrogen per mole of febrifugine. The catalyst was filtered off, the solution evaporated to dryness at reduced pressure and room temperature, and the crystalline residue recrystallized from hot ethanol to yield a first crop of 263 mg. (64%) of colorless crystals melting at 187–192° and a second crop of 34 mg. melting at 163–184°. The mother liquors taken to dryness gave 107 mg. of oil. The dihydrofebrifugine was recrystallized from ethanol to constant melting point, 192–193°.

Anal. Calcd. for $C_{16}H_{21}O_3N_3$: C, 63.3; H, 7.0; N, 13.9. Found: C, 63.3; H, 7.0; N, 14.1.

Dihydroisofebrifugine.—The same method was used as given for dihydrofebrifugine. A 400-mg. sample of isofebrifugine took up the theoretical amount of hydrogen in three hours. The residue on evaporation of the solvent was an oil which was crystallized from acetone to give a first crop amounting to 182 mg. (45%), m.p. 151–154°. A second crop from acetone–benzene amounted to 97 mg., m.p. 86–124°. The mother liquors, evaporated to dryness, left 127 mg. of oil. The dihydroisofebrifugine was recrystallized from acetone to constant melting point, 156.5–157.5°.

Anal. Calcd. for $C_{16}H_{21}O_3N_3$: C, 63.3; H, 7.0; N, 13.9. Found: C, 63.4; H, 6.9; N, 13.8.

Oxidation.—The alkaloids are relatively stable toward acid permanganate, but both are oxidized rapidly by alkaline permanganate to give good yields of 4-quinazolone. Either alkaloid is oxidized rapidly by periodate in neutral or slightly alkaline solution to give the same crystalline product. Although this periodate oxidation product has not been fully characterized and will be dealt with in a later paper, the analyses suggest that it is formed from either alkaloid by the loss of two hydrogens.

Alkaline Permanganate Oxidation.—An aqueous solution of 160 mg. of febrifugine dihydrochloride made alkaline with potassium hydroxide was treated dropwise with a saturated solution of potassium permanganate until the rapid decolorization had ceased. After centrifugation, the supernatant was neutralized with hydrochloric acid and evaporated to dryness. The residue was extracted with absolute ethanol, and the soluble portion recrystallized from ethanol and acetone to give 40 mg. of 4-quinazolone, m.p. 212–213°, identified by absorption spectra and mixed m.p. with a sample prepared by the method of Niementowski.[20]

Anal. Calcd. for $C_8H_6ON_2$: C, 65.7; H, 4.1; N, 19.2. Found: C, 65.3; H, 4.1; N, 19.0.

Oxidation of isofebrifugine under similar conditions gave the same result.

Periodate Oxidation.—A 270-mg. sample of isofebrifugine was dissolved in 30 ml. of water, and 150 ml. of 0.026 *N* disodium paraperiodate was added. After one hour, the solution was saturated with carbon dioxide and extracted with 150 ml. of chloroform in five equal portions. The chloroform solution was dried over anhydrous sodium sulfate and evaporated at reduced pressure. There was obtained 103 mg. of crude solid which, after several crystallizations from ethanol, gave 72 mg. of colorless clusters of needles, m.p. 165–166° (dec.).

Anal. Calcd. for $C_{16}H_{17}O_3N_3$: C, 64.2; H, 5.7; N, 14.0. Found: C, 64.4; H, 6.0; N, 13.7.

Febrifugine oxidized under the same conditions gave the same product.

Hydrolysis.—Both alkaloids are very stable toward acid hydrolysis. It was possible to isolate unchanged febrifugine from a solution of the alkaloid in 70% sulfuric acid after heating at 100° for one hour.

Both alkaloids are very susceptible to alkaline hydrolysis and at room temperature overnight in 2.5 *N* sodium hydroxide either is hydrolyzed to give a good yield of anthranilic acid, a low yield of ammonia, and formic acid. Oils and resins of unknown constitution also result on

alkaline hydrolysis, and these when heated give a positive pine splinter test.

Dihydrofebrifugine also is easily hydrolyzed under alkaline conditions, and such a hydrolysis, does not result in the formation of oils and resins, nor of ammonia, although anthranilic acid and formic acid are formed. It has been possible to isolate from such a hydrolyzate a benzenesulfonyl derivative with the tentative formula $C_8H_{13}ON_2(C_6H_5SO_2)_3$.

Alkaline Hydrolysis of the Alkaloids.—Several hydrolyses were carried out by allowing *ca.* 100-mg. samples of the alkaloids to stand one to two days at room temperature in 2.0 ml. of 2.5 *N* sodium hydroxide. In two experiments a closed system was used, nitrogen was passed through the solutions, and the effluent gas was passed through a known amount of standard hydrochloric acid. Back-titration indicated the formation of 0.044 milliequivalent of volatile base from 0.30 millimole of isofebrifugine and 0.015 milliequivalent from 0.27 millimole of febrifugine. That the volatile base was ammonia was confirmed by evaporating the titration solutions and preparing the characteristic octahedra of ammonium chloroplatinate.[22]

The alkaline hydrolyzates were next brought to *p*H 8–9 by adding hydrochloric acid, and the mixtures extracted with 1:4 butanol–chloroform. These extracts on drying and evaporating to dryness yielded about 20% of the original weight of alkaloid as a dark brown oil, soluble in dilute hydrochloric acid and precipitated from the acid solution by base. Attempts to prepare the acetyl derivative, the picrate and the chloroplatinate led to amorphous, colored precipitates. When this material was heated with 20% aqueous sodium hydroxide, a blob of resin collected rapidly. When this resin was heated in a dry test-tube, the vapors above it gave a pine splinter test for pyrrole.

The hydrolyzate was next acidified with hydrochloric acid to *p*H 3–4, and the mixture was extracted with chloroform. This chloroform extract when taken to dryness gave a light crystalline solid amounting to *ca.* 40% of the original weight of the alkaloid. It was sublimed, recrystallized from aqueous alcohol and proved to be anthranilic acid (m.p. 145–146° with no depression in mixed melting point with an authentic sample).

The aqueous phase from these extractions was next taken to dryness in a vacuum desiccator over sodium hydroxide. This residue if warmed with 20% aqueous sodium hydroxide gave very rapidly a blob of resin which gave a pine splinter test for pyrrole when heated with zinc dust. Attempts to prepare an acetyl derivative, a picrate, a chloroplatinate, an oxime and a phenacyl ester from this residue were unsuccessful.

A little of the original hydrolyzate when treated with magnesium in hydrochloric acid, and then warmed with chromotropic acid in 72% sulfuric acid gave a violet color, indicating the presence of formic acid.[23]

Alkaline Hydrolysis of Dihydrofebrifugine.—When 311 mg. of dihydrofebrifugine was allowed to stand with 4.0 ml. of 2.5 *N* sodium hydroxide for two days it slowly went into solution. The solution was then acidified with hydrochloric acid to *p*H 3–4, and extracted with chloroform to remove anthranilic acid (a previous experiment had shown that under these conditions a 75% yield of anthranilic acid was isolated). The solution was then evaporated on a steam-cone with a stream of air until the volume was *ca.* 5 ml. One-half gram of sodium hydroxide and 1 ml. of benzenesulfonyl chloride were added and the mixture shaken vigorously, with cooling. The solid which separated was crystallized from acetonitrile to give 98 mg. of colorless crystals which were recrystallized to constant melting point, 211–213°.

(22) Chamot and Mason, "Handbook of Chemical Microscopy," John Wiley and Sons, Inc., New York, N. Y., 1931, Vol. II, pp. 75–76.

(23) F. Feigl, "Spot Tests," Elsevier Press, New York, N. Y., 3rd. ed., p. 397.

Anal. Calcd. for $C_{26}H_{28}O_7S_3N_2$: C, 54.2; H, 4.9; S, 16.7; N, 4.9. Found: C, 54.3; H, 5.1; S, 16.7; N, 5.1.

Discussion of Results

With the exception of the alkaloids of the Cinchona type occurring in certain species of the Rubiaceae, those isolated from plants used in some of the native remedies for malaria have generally proven to be without plasmocidal activity. The alkaloids isolated from *D. febrifuga* are therefore of particular interest not only because they are the first to be found in a plant of the Saxifragaceae but also because one of them has high plasmocidal activity.

In avian tests the antimalarial activity of various samples of *D. febrifuga* leaf material has consistently been about twice that of the roots. However, we have always found five to ten times more febrifugine in root than in leaf material. The amount of febrifugine actually isolated was sufficient to account for *ca.* 25% of the activity of the roots but only 2% of the activity of the leaf material. These discrepancies, even though the bioassay cannot be relied on to better than a factor of two, are still so great as to suggest the presence, at least in leaf material, of some active principle other than febrifugine and isofebrifugine. This possibility is being investigated.

With respect to the relative quantities of febrifugine and isofebrifugine which may be present in *D. febrifuga*, we can only point out that we have consistently isolated from various samples of both root and leaf material more febrifugine than isofebrifugine, and in view of the interconvertibility of the alkaloids it is quite possible that there is little or no isofebrifugine in the intact plant.

Our analytical data for the free bases and their derivatives indicate that the empirical formula for febrifugine and isofebrifugine is $C_{16}H_{19}O_3N_3$. The formation of 4-quinazolone on oxidation, the formation of anthranilic and formic acids on hydrolysis, the ease of hydrolysis (atypical of 3-unsubstituted-4-quinazolones), and the striking agreement in absorption spectra leave little doubt that the aromatic portion of the molecules is a 3-substituted-4-quinazolone. The isolation of a C_8-fragment containing two nitrogen atoms from the hydrolyzate of dihydrofebrifugine not only confirms this conclusion but suggests that the 4-quinazolone nucleus is substituted only at the 3-position.

The quinazolone nucleus accounts for the function of two of the nitrogen atoms and one of the oxygen atoms in the alkaloids. There is reasonably good evidence that the third nitrogen atom forms a secondary aliphatic amine, for both alkaloids have basic groups, pK_B *ca.* 6, both form non-basic, non-acidic dibenzenesulfonyl derivatives, and both form N-nitroso derivatives.

The function of the remaining two oxygen atoms is less clearly indicated. The formation of an oxime and a semicarbazone indicate the presence

of a keto-group at least in febrifugine. The formation of dibenzenesulfonyl derivatives may indicate the presence of an alcoholic hydroxyl group. One benzenesulfonyl group is presumably attached to the secondary amine nitrogen, and the other to one of two atoms, either an oxygen which was originally a hydroxyl group or to the 1-nitrogen of the quinazolone ring. This latter possibility is indicated by the fact that the dibenzenesulfonyl derivatives are insoluble in dilute hydrochloric acid, although the 1-nitrogen of normal 4-quinazolones is sufficiently basic to render them soluble in dilute acid. The involvement of the 1-nitrogen atom in the formation of such a derivative is known in the case of rutacarpine, which forms a monoacetyl and a monobenzoyl derivative.[24]

Alkaloids containing a quinazolone ring system are comparatively rare, the only two previously known being rutacarpine and evodiamine. Furthermore, if as we believe the 4-quinazolone nucleus is attached only through its nitrogen at the 3-position to the other basic portion of the molecule, it will constitute a novel structural feature in alkaloidal chemistry. Lastly, febrifugine and isofebrifugine represent the first instance known to us in which two optically active isomeric alkaloids are so readily interconvertible.

It is evident from published reports that, quite independently of ourselves, investigators both here and abroad have been engaged on the chemistry of *D. febrifuga*. Thus Chou, *et al.*,[25] report the isolation of *three isomeric* alkaloids, α-, β- and γ-dichroines. Despite some disagreement in melting points it is evident that the α-dichroine of the Chinese investigators corresponds to our isofebrifugine, but contrary to their interpretation we believe their own physical data clearly indicate that their β- and γ-dichroines are not two different isomeric alkaloids but correspond to the two crystalline modifications of our febrifugine. Investigators[26] in this country have, without assigning names or mentioning the dimorphism of the higher melting alkaloid, reported results in substantial agreement with ours.

It would appear that some confusion has been occasioned by the way in which investigations of *D. febrifuga* in China have been reported.[25,27,28,29,30] We selected names derived from the plant species to avoid possible confusion with the "dichroin" of Hartwich[10] or the names "Dichroine A" and "Dichroine B" used by Jang, *et al.*,[27] for two incorrectly characterized alkaloids.[29,30] To avoid causing further confusion in the literature we prefer to adhere to the names febrifugine and isofebrifugine since they were the first to be proposed in a publication[6] which gives an adequate characterization of two isomeric alkaloids obtained from *D. febrifuga*.

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Summary

Two interconvertible, isomeric, crystalline, optically active alkaloids, febrifugine and isofebrifugine, have been isolated from the roots and leaves of *Dichroa febrifuga*. Febrifugine is a powerful antimalarial, having about 100 times the activity of quinine against bird malaria.

An empirical formula, $C_{16}H_{19}O_3N_3$ is proposed, and evidence is given which indicates that structurally these alkaloids are 4-quinazolones substituted only on the 3-nitrogen.

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