The column was washed with water. In all, 30 ml. of eluate was collected and lyophilized.

The solid product was triturated with boiling chloroform, and the chloroform solution filtered. Concentration of the filtrate to a small volume gave crystals which were filtered and washed with cold chloroform. The yield of crystalline product, m. p. 75–77°, was 35 mg. Recrystallization of the product from chloroform gave pure dihydrodesoxystreptose, m. p. 78–79°.

Anal. Calcd. for $C_6H_{12}O_4$: C, 48.64; H, 8.17. Found: C, 48.53; H, 7.96.

Periodate Oxidation of Dihydrodesoxystreptose.—A solution of 15.2 mg. (0.102 millimole) of dihydrodesoxystreptose and 55.3 mg. (0.259 millimole) of sodium periodate in 10 ml. of water was allowed to stand at room temperature for one hour. Titrations of aliquot portions showed the presence of 0.047 millimole of periodate and of 0.098 millimole of acid, corresponding to a consumption of 2.07 equivalents of periodate with formation of 0.96 equivalent of acid.

In another experiment, 6.7 mg. of dihydrodesoxystreptose in 2 ml. of water containing 14 mg. of sodium bicarbonate was oxidized with 19.3 mg. (2 equivalents) of sodium periodate. After one hour, no periodate could be detected, and a solution of 81 mg. of dimedone in 2 ml. of ethanol was added. The crystalline precipitate which formed rapidly was collected, washed with 50% ethanol and dried. It weighed 11.6 mg. (88%) and melted at 182–189°. One recrystallization of this material from dilute ethanol gave pure dimedone–formaldehyde condensation product, m. p. and mixed m. p. 191–194°.

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Summary

Treatment of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal with Raney nickel catalyst has given two products, tetraacetyl-bis-desoxystreptobiosamine and tetraacetyldesoxystreptobiosamine.

The additional oxygen atom of the desoxy compound is present as a glycosidic hydroxyl group. Tetraacetyldesoxystreptobiosamine was characterized by the preparation of N-acetyldesoxystreptobiosamine, pentaacetyldesoxystreptobiosamine and methyl tetraacetyldesoxystreptobiosaminide.

Acid hydrolysis of tetraacetyl-bis-desoxystreptobiosamine yielded N-methyl-L-glucosamine and bis-desoxystreptose.

Bis-desoxystreptose has been determined by periodic acid oxidation studies to be a 3,4-dihydroxy-2,3-dimethyltetrahydrofuran. The two hydroxyl groups of bis-desoxystreptose appear to be in a *cis* configuration. The structure of tetraacetyl-bis-desoxystreptobiosamine is given.

Pentaacetyldihydrodesoxystreptobiosamine was hydrolyzed with acid to give dihydrodesoxystreptose. The periodate degradation of dihydrodesoxystreptose to yield formaldehyde and an acid was in agreement with the proposed structure of this product, and offered further evidence for the position of the linkage of streptidine to streptobiosamine.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

Alkaloids of *Dichroa Febrifuga* Lour.

BY FREDERICK A. KUEHL, JR., CLAUDE F. SPENCER AND KARL FOLKERS

The tests on extracts of about six hundred plants have shown that several plants possess interesting unknown principles which exibit antimalarial activity.[1] Of those plants containing active principles which were investigated, *Dichroa febrifuga* Lour. was particularly interesting because of the high antimalarial activity of the alkaloidal fraction isolated from it. Extractions were made on dried roots, stems and leaves of the plant obtained from both India and China. Unfortunately, the samples of *Dichroa febrifuga* Lour. from India, the material available when most of this work was done, contained only about one-tenth of the alkaloidal fraction present in the Chinese samples.

A number of extraction procedures was investigated. The best yields of the alkaloidal fraction from the Chinese root material were 0.1 to 0.15%. The yield of alkaloids from stem and leaf material was invariably much lower.

(1) Spencer, Koniuszy, Rogers, Shavel, Easton, Kaczka, Kuehl, Phillips, Walti and Folkers, *Lloydia*, **10**, 145 (1947).

Nothing crystallized directly from the crude alkaloidal fraction. A solution of this material and oxalic acid, however, gave a characteristic crystalline oxalate, which represented more than 75% of the antimalarial activity of the crude fraction. The yield of crude oxalate was 0.05% from the Chinese root sample and 0.005% from the Indian root sample. The recrystallized oxalate, m. p. *ca.* 212–214° (dec.), $(\alpha)^{25}D$ +18° (*c*, 1.5 in water), had a composition which was in agreement with the formula $(C_{16}H_{19}N_3O_3)_2 \cdot C_2H_2O_4$.

When a sample of a recrystallized oxalate was converted to the free base, two different crystalline alkaloids appeared which were not separated satisfactorily by crystallization. Chromatography of the mixture over alumina gave crystalline alkaloid I, m. p. 131–132°, $(\alpha)^{25}D$ +31° (*c*, 1.5 in ethanol), $(\alpha)^{25}D$ +120° (*c*, 0.8 in chloroform) and the properties of this alkaloid were not changed after repeated crystallization. The results of analytical data, potentiometric titration and ebullioscopic molecular weight determination were in-

dicative of the formula $C_{16}H_{19}N_3O_3$ for this alkaloid.

A sample of alkaloid I was chromatographed over a mixture of Norite and filter paper pulp. The specific rotation of all twenty eluates was about the same, but the value was somewhat lower than that of the starting material. After the eluates were allowed to stand for two days, they acquired a yellow color. The combined fractions 1–16 yielded alkaloid I upon crystallization, and the mother liquor yielded alkaloid II, m. p. 140–142°, $(\alpha)^{17}D$ +21° (*c*, 1.4 in ethanol). The composition of alkaloid II was also indicative of the formula $C_{16}H_{19}N_3O_3$.

These results were interpreted as indicating that alkaloid I is unstable under certain conditions and may be converted to some extent into alkaloid II. To test for this reaction or conversion, an alcoholic solution of alkaloid I was refluxed for twleve hours, and it was observed that the specific rotation decreased and approximated that of alkaloid II. In contrast, the specific rotation of alkaloid II was unchanged after this treatment. In the extraction of the plant material, where the alkaloid undoubtedly exists as a salt, alkaloid I was isolated after the root was extracted for three days with hot methanol. It is of interest that despite the instability of alkaloid I in solution, reference samples of the crystalline alkaloid have been on hand for almost three years without evidence of change.

Not only do alkaloids I and II appear to have the same formula $C_{16}H_{19}N_3O_3$, they also have indistinguishable ultraviolet and infrared absorption spectra. The ultraviolet absorption spectrum of alkaloid I in ethanol solution showed maxima at 2250 Å. (*E*% 900), 2650 Å. (*E*% 267), 3020 Å. (*E*% 125), and 3140 Å. (*E*% 101); the spectrum of alkaloid II showed maxima at 2250 Å. (*E*% 895), 2660 Å. (*E*% 246), 3010 Å. (*E*% 109), and 3130 Å. (*E*% 98). The infrared absorption spectra of both compounds showed bands at 6.03, 6.26, 6.45 and 6.78 μ.

We are indebted to Dr. A. O. Seeler and Miss Christine Malanga of the Merck Institute for Thereapeutic Research for the antimalarial tests. The crude alkaloidal fraction at a level of 20 mg./kg. orally showed an activity equivalent to a dose of 40 mg./kg. of quinine for essentially complete suppression of the trophozoite-induced infections of *Plasmodium gallinaceum* in chicks according to the described procedure.[2,3] Alkaloid I at a level of 5 mg./kg. and alkaloid II at a level of 2.5 mg./kg. orally were equivalent to a dose of 40 mg./kg. of quinine. The toxicity of these alkaloids to chicks was rather high since doses only twice the above-mentioned levels were toxic. A communication[4] has appeared which announced the isolation of two alkaloids from *Dichroa febrifuga* Lour. The melting points, compositions, and ultraviolet absorption spectra of these two alkaloids correspond closely with the properties of the two alkaloids which we have isolated. Our supply of alkaloids, as well as plant material, is exhausted and further comparisons are not possible at present. It was also mentioned[4] that isofebrifugine (alkaloid I) was converted to febrifugine (alkaloid II) by heat. Febrifugine showed approximately one hundred times the activity of quinine and isofebrifugine showed relatively slight activity against *P. lophurae* in ducks.

We also found[4] no evidence of the two alkaloids dichroine A and dichroine B which were described[5] as being derived from *Dichroa febrifuga* Lour. (Ch'ang Shan).

Experimental

Crystalline Alkaloidal Oxalate from *Dichroa febrifuga* Lour. from China.—A 2467-g. portion of finely ground root material of *Dichroa febrifuga* Lour., W. M. Clark 18634,[6] was moistened with water and extracted in a Soxhlet with methanol for three days. The methanol extract was evaporated *in vacuo*, and the aqueous solution remaining (*ca.* 800 ml.) was adjusted to *p*H 3 with dilute hydrochloric acid. The aqueous solution was extracted continuously with chloroform for twenty hours to remove impurities, and then the solution was adjusted to *p*H 8 with sodium bicarbonate and the alkaloids were removed by continuous chloroform extraction for twenty hours. The alkaloidal fraction was obtained as a brown residue, 3.78 g. (0.15%), after removal of the chloroform. It was dissolved in 25 ml. of 50% methanol and oxalic acid was added to *p*H 3. The resulting solution was warmed, filtered and concentrated. The gummy residue was dissolved in methanol and treated with acetone, and 1.453 g. (0.052%) of a crystalline oxalate, m. p. 199–201°, was obtained. After recrystallization from 50% methanol, the oxalate melted at 215–218° (dec. temperature somewhat dependent upon the rate of heating), $(\alpha)^{25}D$ +17° (*c*, 1.0 in water).

Extraction of *Dichroa febrifuga* Lour. from India.—An alternative extraction procedure to obtain the alkaloidal fraction was carried out as follows. A 4901-g. portion of ground root of *Dichroa febrifuga* Lour., National Institute of Health (Kaleshan) 18629, was moistened with water, added to *ca.* 15 l. of methanol and then the mixture was boiled for three hours. The extract was removed by filtration and the extraction was repeated two more times with fresh solvent. The combined extract was concentrated *in vacuo* to a volume of *ca.* 2 l. and the solution was adjusted to *p*H 8 and continuously extracted with chloroform. After distillation of the chloroform, the residue weighed 9.845 g. It was dissolved in 150 ml. of chloroform and the solution was extracted with 5–40 ml. portions of 3.5% hydrochloric acid. The aqueous extract was then adjusted to *p*H 8 and continuously extracted with chloroform to give 1.424 g. (0.029%) of the alkaloidal fraction.

Crystalline Alkaloidal Oxalate from *Dichroa febrifuga* Lour. from India.—Two kilograms of ground root material of *Dichroa febrifuga* Lour., Biswas 18637, was extracted in a Soxhlet and the methanol extract was processed in the manner described above. The alkaloidal fraction yielded 98 mg. of crude oxalate, m. p. 195–210°. After two recrystallizations from 50% methanol, the oxalate melted at 213–214° (dec.), $(\alpha)^{25}D$ +18° (*c*, 0.5 in water).

Anal. Calcd. for $(C_{16}H_{19}N_3O_3)_2 \cdot C_2H_2O_4$: C, 58.85; H, 5.83; N, 12.10. Found: C, 58.97; H, 6.06; N, 12.37.

(2) Seeler, Malanga and Pierson, *Proc. Soc. Exp. Biol. Med.*, **59**, 291 (1945).

(3) Seeler and Malanga, *ibid.*, **63**, 194 (1946).

(4) Koepfli, Mead and Brockman, THIS JOURNAL, **69**, 1837 (1947).

(5) Jang, Fu, Wang, Huang, Lu and Chou, *Science*, **103**, 59 (1946).

(6) The collector's names and specimen numbers were assigned by Mr. B. A. Krukoff.

Alkaloids I and II from the Oxalate from a Chinese Sample.—An aqueous solution of 4.38 g. of the oxalate, $(\alpha)^{25}D$ +17° (*c*, 1.0 in water), was treated with sodium bicarbonate to *p*H 8, and extracted continuously for three hours with chloroform. The chloroform extract was concentrated to a residue *in vacuo*. An alcohol solution of this residue deposited 2.74 g. of crystalline alkaloid I, m. p. 131–132° (softening at 127°), $(\alpha)^{25}D$ +33.6° (*c*, 1.7 in alcohol). On standing, the mother liquor deposited crude crystalline alkaloid II, m. p. 135–142°, $(\alpha)^{25}D$ +21° (*c*, 0.9 in ethanol).

Upon recrystallization of the first crop, two distinct fractions were obtained, the first crystallizing immediately in small needles, 1.700 g., m. p. 124–130°, $(\alpha)^{25}D$ +31° (*c*, 1.1 in alcohol), and the second crystallizing in long needles on standing overnight, 0.359 g., m. p. 135–142°, $(\alpha)^{25}D$ +23° (*c*, 0.9 in alcohol).

Separation of Alkaloid I and II by Chromatography.—A solution of 2.487 g. of a mixture of the two alkaloids in 25 ml. of chloroform was passed through a column containing 35 g. of acid-washed alumina. Elution with 800 ml. of chloroform gave 1.604 g. of residue, $(\alpha)^{25}D$ +31° (*c*, 2.6 in ethanol). A second elution with 200 ml. of chloroform containing 5% methanol gave 749 mg. of residue, $(\alpha)^{25}D$ +21° (*c*, 2.0 in ethanol). Finally, elution with 200 ml. of methanol gave 106 mg. of material, $(\alpha)^{25}D$ +20° (*c*, 1.3 in ethanol). The first eluted fraction, after recrystallization from benzene, gave 376 mg. of alkaloid I, m. p. 131–132°, $(\alpha)^{25}D$ +31° (*c*, 1.5 in ethanol). Concentration of the mother liquor yielded a second crop of 0.984 g., m. p. 124–130°. Recrystallization of this second crop gave 0.775 g., m. p. 128–132°, $(\alpha)^{25}D$ +31° (*c*, 1.9 in alcohol). This was combined with the first crop and recrystallized again from benzene, giving 0.829 g., m. p. 131–132°, $(\alpha)^{25}D$ +31° (alcohol), $(\alpha)^{25}D$ +120° (*c*, 0.8 in chloroform). The melting point of this material did not alter after further recrystallization from methanol.

Anal. Calcd. for $C_{16}H_{19}N_3O_3$: C, 63.8; H, 6.4; N, 14.0, mol. wt., 301. Found: C, 63.91; H, 6.50; N, 14.10, eq. wt. (potentiometric titration) 314; mol. wt., 281 (ebullioscopic in benzene).

Chromatography of Alkaloid I.—A solution of 1.6 g. of alkaloid I, $(\alpha)^{25}D$ +33.6° (*c*, 1.7 in ethanol), in 5 ml. of ethanol was diluted with chloroform and the solution was concentrated *in vacuo* to a gum. This gum was dissolved in chloroform and the concentration was repeated to yield an alcohol-free gum. A solution of this gum in 10 ml. of chloroform was poured onto a column composed of 25 g. of Norite and 2 g. of pulverized filter paper. The column was developed with 200 ml. of chloroform and then eluted with chloroform containing 2% ethanol. The data are shown in Table I.

The eluates were allowed to stand for two days, during which time they acquired a yellow color. Fractions 4, 6, 8, 10 and 12 were combined to give 0.435 g. of residue, a total of 1.24 g. in all (78%). Fractions 1 to 16 were combined and concentrated *in vacuo* to a residue, which was dissolved in a small amount of alcohol and decolorized with a little charcoal. Upon cooling, 560 mg. of alkaloid I deposited as colorless needles, m. p. 131–132°, $(\alpha)^{25}D$ +32° (*c*, 1.7 in ethanol). After standing for several days, the mother liquor deposited 170 mg. of alkaloid II. After several recrystallizations, alkaloid II melted at 140–142°, $(\alpha)^{25}D$ +21° (*c*, 1.4 in ethanol).

Anal. Calcd. for $C_{19}H_{16}N_3O_3$: C, 63.80; H, 6.40; N, 14.00. Found: C, 63.42; H, 6.56; N, 14.33.

TABLE I

CHROMATOGRAPHIC DATA ON ALKALOID I

Fraction	Volume, ml.	Weight of residue, mg.	$[\alpha]^{25}D$ (in ethanol)
1	10	11	+29°
2	10	38	
3	10	65	+30°
4	10		
5	10	85	+29°
6	10		
7	10	78	+31°
8	10		
9	10	83	+29°
10	10		
11	10	84	+32°
12	10		
13	20	85	+31°
14	30	57	+32°
15	30	37	+31°
16	50	45	+29°
17	50	41	+27°
18	50	35	+26°
19	50	28	+26°
20	50 (methanol)	29	+23°

Stability Tests in Ethanol.—A solution of 22 mg. of alkaloid I, m. p. 130–132°, $(\alpha)^{25}D$ +32° (*c*, 1.7 in ethanol), in 25 ml. of ethanol was boiled for twelve hours. The solvent was then removed *in vacuo*. The residue showed $(\alpha)^{25}D$ +24° (*c*, 1.5 in ethanol).

A solution of 23 mg. of alkaloid II, m. p. 140–142°, $(\alpha)^{25}D$ +20.5° (*c*, 1.4 in ethanol) in 25 ml. of ethanol was boiled for twelve hours. The residue showed $(\alpha)^{25}D$ +20° (*c*, 1.5 in ethanol).

Preparation of the Oxalate of Alkaloid I.—A mixture of 33 mg. of alkaloid I, m. p. 131–132°, $(\alpha)^{25}D$ +31° (*c*, 1.1 in ethanol) and 14 mg. of oxalic acid was heated in 1 ml. of 50% methanol until the components dissolved. After standing in ice, 19 mg. of the oxalate salt deposited which, when recrystallized from 50% methanol to constant melting point, was obtained as colorless crystals, m. p. 212–213° (dec.), $(\alpha)^{25}D$ +19° (*c*, 0.3 in water).

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Summary

Two crystalline alkaloids have been isolated from *Dichroa febrifuga* Lour. which show antimalarial activity. These two alkaloids appear to be isomeric, both having the composition $C_{16}H_{19}N_3O_3$.

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