58. *The* Erythrophleum *Alkaloids. Part I. Erythrophleine.*

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Erythrophleine, an amorphous alkaloid from the bark of *Erythrophleum guineense* G. Don., appears to have the formula $C_{24}H_{39}O_5N$. Hydrolysis with dilute acids yields crystalline *erythrophleic acid*, $C_{21}H_{32}O_5$, and β-methylaminoethanol. The alkaloid is considered to be the β-methylaminoethyl ester of erythrophleic acid. Erythrophleic acid contains one carbonyl group, one hydroxy- and one methoxy-group. As it contains in addition one double bond, probably in the αβ-position with respect to the carboxyl group, it must contain three rings. Selenium dehydrogenation of the acid yields 1 : 7 : 8-trimethylphenanthrene and a selenium compound, $C_{19}H_{16}Se$, of unknown structure. It is suggested that erythrophleic acid is a diterpene derivative. Since erythrophleine has a digitalis-like action on the heart, it is clear that cardiac activity of this type is not confined, as generally believed, to steroids containing unsaturated lactone rings.

Trees of the genus *Erythrophleum* belonging to the order *Leguminosae* have long been known to contain alkaloids possessing a remarkable action on the heart which is similar to that exhibited by the cardiac glycosides and toad poisons. Of the species examined, *Erythrophleum guineense* G. Don. is perhaps the commonest. This tree, known by a variety of names (*e.g.*, sassy or redwater tree), is widely distributed in West and Central Africa, where the bark is used by native tribes as a source of ordeal and arrow poisons. The first scientific investigations of the bark were made by Gallois and Hardy (*Compt. rend.*, 1875, **80**, 1221; *J. Pharm. Chim.*, 1876, **24**, 25; *Bull. Soc. chim.*, 1876, **26**, 39), who isolated from it an amorphous, highly poisonous alkaloid, erythrophleine, with a digitalis-like action on the heart. Erythrophleine was later investigated by Harnack and Zabrocki (*Arch. exp. Path. Pharmakol.*, 1882, **15**, 403), but little emerged save that the base, con-

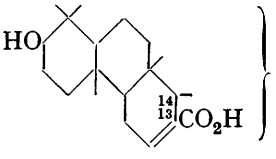
sidered to have a formula $C_{28}H_{43-45}O_7N$, was decomposed by boiling with hydrochloric acid, yielding an amorphous nitrogen-free acid, and a base of low molecular weight believed to be methylamine. Harnack employed for his investigations erythrophleine supplied by E. Merck and Co., Darmstadt. Later investigations on *E. guineense* by Power and Salway (*Amer. J. Pharm.*, 1912, **84**, 337) and by Maplethorpe (*Pharm. J.*, 1923, **111**, 85) confirmed the presence of erythrophleine as described by Harnack.

Our interest in erythrophleine was aroused some years ago partly because of its cardiac activity, unique among alkaloids, and partly because of the curious behaviour towards acid reported by Harnack (*loc. cit.*), and a series of investigations was commenced, the starting material being the amorphous erythrophleine sulphate obtainable from E. Merck and Co., Darmstadt, whose source is believed to be *E. guineense*. For various reasons these investigations were interrupted for a time and have only recently been taken up again. We now wish to place on record the results so far obtained, particularly as a number of publications from other laboratories dealing with *erythrophleum* alkaloids have recently appeared. Dalma (*Ann. Chim. Appl.*, 1935, **25**, 569) isolated from *E. guineense* three crystalline alkaloids, namely, cassaine, $C_{24}H_{39}O_4N$, cassaidine, $C_{24}H_{43}O_5N$, and norcassaidine, $C_{23}H_{41}O_5N$, and one amorphous alkaloid, homophleine, $C_{56}H_{88}O_9N_2$. More recently the same author (Atti X Intern. Congr. Chim. Rome, 1938) reported the isolation from *E. couminga* of two alkaloids, coumingine, $C_{28}H_{45}O_6N$, and coumingaine, $C_{32}H_{51}O_8N$, the latter being amorphous. None of these alkaloids appears to be identical with erythrophleine; their pharmacological properties have been investigated by a number of workers, all of whom agree that they exhibit in varying degree digitalis-like action and local anæsthetic properties. According to Chen, Hargreaves, and Winchester (*J. Amer. Pharm. Assoc.*, 1938, **27**, 9) all the new alkaloids with the exception of coumingine are less powerful cardiac poisons than erythrophleine.

Erythrophleine sulphate *puriss.* as supplied by E. Merck and Co. is in our experience a cream-coloured amorphous powder of fairly constant composition. So far we have been unable to crystallise either the free base or any of its salts. Since no separation into distinct fractions has been observed during any of our experiments, we are of the opinion that the commercial product consists essentially of one compound, although it is difficult to advance proof of homogeneity in view of its amorphous nature. This view of the individuality of erythrophleine is in accord with that of earlier investigators.

Assignment of a definite formula to erythrophleine solely on the basis of elementary analysis of its amorphous sulphate would not be possible, but the analytical figures are in fair agreement with the formula $C_{24}H_{39}O_5N$, which best explains its chemical behaviour. In agreement with earlier observations the base was found to undergo ready hydrolysis with acids or alkalis. The products of hydrolysis, obtained in satisfactory yield, were a crystalline acid, $C_{21}H_{32}O_5$, to which we apply the name *erythrophleic acid*, and a steam-volatile base. The latter, isolated as its *picrate*, was identified as β-methylaminoethanol by analysis and by direct comparison of the picrate and of the corresponding *α-naphthylthiourea* with synthetic specimens. These results suggest that erythrophleine (I) is the β-methylaminoethyl ester of erythrophleic acid.

(I.) $C_{20}H_{31}O_3\cdot CO\cdot O\cdot CH_2\cdot CH_2\cdot NHMe$



(II.)

Such a structure readily accounts for the local anæsthetic properties of the alkaloid, but it is rather surprising to find digitalis-like properties in an ester of this nature. Hitherto cardiac activity of this type has always been associated with the presence of an unsaturated lactone ring in a steroid molecule. Dr. K. K. Chen (Indianapolis), to whom we are much indebted, carried out pharmacological tests on erythrophleic acid and found that it was entirely devoid of cardiac activity. Quite recently Faltis and Holzinger (*Ber.*, 1939, **72**, 1443) showed that Dalma's cassaine is hydrolysed under conditions similar to those used for erythrophleine, yielding cassaic acid, $C_{20}H_{30}O_4$, and β-dimethylamino-

ethanol and they effected a partial synthesis of the alkaloid by recombining these two components as an ester. The parallel with erythrophleine is striking, and it seems possible that all the *Erythrophleum* alkaloids may prove to be similar in type.

Erythrophleic acid is a monobasic ketonic acid; of the two oxygen atoms unaccounted for by the carbonyl and the carboxyl group, one is present in a hydroxyl group and the second in a methoxyl group. It yields an oily *methyl* ester, from which a crystalline 2 : 4-*dinitrophenylhydrazone* can be prepared. Acyl derivatives, although undoubtedly formed, have not yet been crystallised. Catalytic hydrogenation indicates the presence of one double bond in erythrophleic acid (and in erythrophleine) and the absorption spectrum (max., *ca.* 2210 A.; log ε, 4·2) indicates that the double bond, although not conjugated with the carbonyl group, is probably in the αβ-position with respect to the carboxyl group. These results indicate that erythrophleic acid contains three carbon rings. The acid is thus similar in many respects to cassaic acid, which according to Faltis and Holzinger (*loc. cit.*) is a mono-unsaturated hydroxy-ketonic acid containing three rings.

Further insight into the structure of erythrophleic acid was obtained by dehydrogenation. When it was heated with selenium at 300—320°, two crystalline products were obtained, a hydrocarbon, $C_{17}H_{16}$, and a selenium compound, $C_{19}H_{16}Se$. The former substance was the sole crystalline product on dehydrogenation with palladised charcoal. The hydrocarbon, which had m. p. 143—144°, was identified as 1 : 7 : 8-trimethylphenanthrene by direct comparison with a synthetic specimen of this compound. The two substances showed no depression in m. p. on mixing; the identity was confirmed by comparison of their picrates and trinitrobenzene derivatives. The isolation of 1 : 7 : 8-trimethylphenanthrene together with the presence of one carboxyl and one methoxyl group leaves only two carbon atoms of erythrophleic acid unaccounted for; whether the methyl groups occupy the same positions in the parent acid as they do in the dehydrogenation product cannot be definitely stated at present. The formation of the substance $C_{19}H_{16}Se$, although unexpected, is interesting; investigation of its structure should throw further light on the orientation of substituents in the erythrophleic acid molecule. Although all the cardiac glycosides belong to the steroid group of compounds, it is clear that erythrophleic acid, and hence also erythrophleine, are probably to be regarded as diterpene derivatives containing a reduced phenanthrene skeleton. 1 : 7 : 8-Trimethylphenanthrene is a characteristic dehydrogenation product of several members of the diterpene group, *e.g.*, *iso*agathenedicarboxylic acid, sclareol, manoyl oxide. The view that it is a diterpenoid being accepted, then, by analogy with known members of the group, structure (II) suggests itself as a tentative formula for erythrophleic acid. In this formula the double bond might equally well be between C_{13} and C_{14} and the positions of the methoxy- and the keto-group remain unknown. The investigation of erythrophleic acid is being continued.

While this paper was being prepared for publication a further communication (Ruzicka and Dalma, *Helv. Chim. Acta*, 1939, **22**, 1516) appeared dealing with cassaine, in which the formation of 1 : 7 : 8-trimethylphenanthrene on selenium dehydrogenation of dihydroxycassanic acid ($C_{20}H_{30}O_4$) is reported, thus further emphasising the close relationship between cassaine and erythrophleine. It seems possible that erythrophleic acid may be a methoxycassaic acid. In the same number of the Swiss journal (p. 1497) Dalma has published an important paper in which differences are reported in the alkaloids isolated from *E. guineense* collected in different localities; *e.g.*, erythrophleine instead of cassaine was isolated from bark derived from the Upper Congo region; it seems at least a possible explanation that the term *E. guineense* as ordinarily used may embrace more than one botanical species.

Experimental.

Erythrophleine Sulphate.—The salt was obtained as a cream-coloured amorphous powder readily soluble in water and in a number of organic solvents, including benzene (Found: C, 60·4; H, 8·2; N, 3·1; S, 2·9; MeO, 6·6; NMe, 4·0. Calc. for $C_{24}H_{39}O_5N,0{\cdot}5H_2SO_4$: C, 61·3; H, 8·3; N, 3·0; S, 3·4; 1 MeO, 6·6; 1 NMe, 6·2%). On catalytic hydrogenation (platinic oxide) 1 mol. of hydrogen was rapidly absorbed and a second very slowly. The substance gave a positive reaction for carbonyl with 2 : 4-dinitrophenylhydrazine.

Hydrolysis of Erythrophleine.—(1) *Erythrophleic acid.* For the preparation of *erythrophleic*

acid in quantity, hydrolysis with dilute sulphuric acid is the most reliable method, although other acids or sodium hydroxide may be employed. The duration of hydrolysis and the strength of acid used are of importance, as prolonged heating with acids tends to give rise to resinous products. The following procedure was adopted as a standard method.

Erythrophleine sulphate (5 g.) is dissolved in N/3-sulphuric acid (100 c.c.), and the solution boiled under reflux during 12 hours. A white crystalline precipitate of erythrophleic acid forms and after cooling is filtered off. The filtrate is again heated for 12 hours, a further crop of acid being obtained; a small additional amount may be recovered from the mother-liquors. The erythrophleic acid thus obtained may be purified by dissolution in dilute sodium hydroxide solution and reprecipitation with hydrochloric acid or by recrystallisation from dilute alcohol. As ordinarily obtained in the above process, the acid forms colourless platelets, m. p. 204—206°, but by repeated crystallisation from alcohol the m. p. may be raised to 218° [Found: C, 69·2, 69·3; H, 8·5, 8·6; MeO, 8·9; *M* (Rast), 389; *M* by silver salt (23·2% Ag), 359. $C_{21}H_{32}O_5$ requires C, 69·2; H, 8·8; 1 MeO, 8·5%; *M*, 364. Zerewitinoff estimations showed 1·5, 1·7 active hydrogen atoms and oxidation with chromic acid (Kuhn–Roth) gave 1·5, 1·5 mols. of acetic acid]. Total yield, 2·5 g. The acid gives a positive reaction for carbonyl with 2 : 4-dinitrophenylhydrazine and on catalytic hydrogenation (micro; platinic oxide catalyst) takes up 1 mol. of hydrogen rapidly (double bond) and a second slowly (carbonyl group). It is readily oxidised by potassium permanganate in alkaline solution, although stable to this reagent at room temperature in acetone solution. The pure acid has $[\alpha]_D^{20°}$ — 40° (c = 0·48 in chloroform; l = 1).

(2) β-*Methylaminoethanol.* Hydrolysis of erythrophleine was effected by heating with N/3-sulphuric acid during 24 hours as above, and, after removal of erythrophleic acid, the solution was evaporated to small bulk and made strongly alkaline with potassium hydroxide, and the mixture distilled over a free flame. The strongly basic distillate was extracted several times with ether, and the ethereal solution dried over potassium carbonate. On addition of ethereal picric acid to a portion of this solution a crystalline *picrate* separated. After two recrystallisations from ethyl acetate the picrate had m. p. 148° (Found: C, 35·7; H, 4·0; N, 18·5. $C_3H_9ON,C_6H_3O_7N_3$ requires C, 35·6; H, 4·0; N, 18·4%), undepressed on admixture with an authentic specimen of β-methylaminoethanol picrate (m. p. 148°). A second portion of the solution was mixed with a small volume of an alcoholic solution of α-naphthyl *iso*thiocyanate, and the mixture slowly concentrated on the steam-bath. The colourless solid separating on cooling had m. p. 125° after recrystallisation from dilute alcohol. N-*Methyl*-N-β-*hydroxyethyl*-N′-α-*naphthylthiourea* (Found: N, 11·2. $C_{14}H_{16}ON_2S$ requires N, 10·8%), prepared from synthetic β-methylaminoethanol, had m. p. 125°, undepressed on admixture with the above compound.

Methyl Erythrophleate 2 : 4-*Dinitrophenylhydrazone.*—Erythrophleic acid is readily esterified by heating with methyl-alcoholic hydrogen chloride or sulphuric acid, or by treatment with diazomethane, but the *ester* could not be crystallised. By sublimation in a high vacuum (10^{-4} mm.) at 140° it was obtained as a white amorphous powder (Found: C, 69·5; H, 8·5. $C_{22}H_{34}O_5$ requires C, 69·8; H, 9·0%). Hydrolysis of a small specimen with 4% methyl-alcoholic potash gave erythrophleic acid, m. p. 218° after one recrystallisation.

The methyl ester reacted readily with 2 : 4-dinitrophenylhydrazine to give a 2 : 4-*dinitrophenylhydrazone* crystallising from alcohol in small orange-red plates, m. p. 219° (Found: C, 60·4; H, 6·7; N, 10·0. $C_{28}H_{38}O_8N_4$ requires C, 60·2; H, 6·8; N, 10·0%). The same compound could be prepared directly from erythrophleic acid by warming with a solution of 2 : 4-dinitrophenylhydrazone in methyl alcohol containing a trace of hydrogen chloride.

Dehydrogenation of Erythrophleic Acid with Selenium.—An intimate mixture of erythrophleic acid (1·5 g.) and powdered selenium (3 g.) in a small round flask fitted with a long air-condenser was heated rapidly in a metal bath till the bath temperature reached 270°. The temperature was raised to 310° during the next 15 mins. and maintained between 300° and 320° for 18 hours. After cooling, the bulb contained a button of metallic selenium, on top of which was a yellowish semi-solid mass. The product was separated from the selenium by dissolution in ether. After evaporation of the ether the residue was subjected to distillation in a vacuum, a horizontal bulb tube being used; two fractions were collected: (A) B. p. 215—225°/12 mm. (bath temp.). On cooling, the distillate solidified completely. Thrice recrystallised from absolute alcohol, it formed colourless plates, m. p. 143—144° (Found: C, 92·8; H, 7·3. Calc. for $C_{17}H_{16}$: C, 92·7; H, 7·3%). A mixed m. p. with synthetic 1 : 7 : 8-trimethylphenanthrene (m. p. 142—143°) kindly supplied by Prof. R. D. Haworth showed no depression. The picrate had m. p. 163—164° and the *s*-trinitrobenzene derivative m. p. 190—191°, undepressed on admixture

with the corresponding derivatives of the synthetic hydrocarbon. (B) B. p. 240—280°/12 mm. (bath temp.). The distillate crystallised on cooling, and on heating sintered at about 140° and was not completely molten until 160°. Twice recrystallised from absolute alcohol, long yellowish prisms were obtained, m. p. 161—162° (Found : C, 70·3; H, 4·9. $C_{19}H_{16}Se$ requires C, 70·4; H, 4·9%). The *substance* gave a strong positive test for selenium (oxidation with nitric acid, followed by reduction to selenium with sulphite) even with minute quantities. Alcoholic solutions, on standing for several weeks exposed to air, slowly deposited a red film of selenium. The substance was readily soluble in ether, but much less soluble in alcohol than 1 : 7 : 8-trimethylphenanthrene; it distilled under reduced pressure without decomposition. From the crystallisation mother-liquors of (B) additional small amounts of 1 : 7 : 8-trimethylphenanthrene were obtained.

The total yield of 1 : 7 : 8-trimethylphenanthrene was 90 mg. and of the selenium compound, m. p. 160—162°, *ca.* 30 mg.

Dehydrogenation of Erythrophleic Acid with Palladised Charcoal.—The acid (1 g.) was heated with palladised charcoal (0·5 g.) at 300—310° (bath temp.) during 7 hours. After cooling, the mixture was extracted with ether, and the extract, after removal of the ether, distilled in a vacuum. At 200—220°/12 mm. (bath temp.) a reddish-yellow oil came over which partly solidified on cooling. Recrystallisation from alcohol yielded as sole crystalline product 1 : 7 : 8-trimethylphenanthrene, m. p. 142—143°, identical with the product from the selenium dehydrogenation. The yield (*ca.* 30 mg.) was poor.

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