176. *The Chemistry of the* Mitragyna *Genus. Part I.*

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The alkaloids of the leaves and bark of several species of *Mitragyna* have been examined as a preliminary to a more intensive chemical study of compounds of this class. Rotundifoline has been extracted from the leaves, and rhyncophylline from the bark, of *M. ciliata*. The identity of rhyncophylline with mitrinermine, suggested by Raymond-Hamet and supported by Barger *et al.* but disputed by Millat, has been established. A new phenolic alkaloid, *mitragynol*, has been isolated from *M. rotundifolia*; its methyl ether has been shown to be identical with dihydrorotundifoline. The hitherto unknown *dextro*-form of mitraphylline has been isolated from *M. rubrostipulaceæ*. Evidence has been obtained that the base, $C_9H_{13}N$, obtained by Barger *et al.* by selenium dehydrogenation of rotundifoline is 3 : 4-diethylpyridine.

Quinovic acid has been extracted from both leaves and bark of several species of *Mitragyna*, and a sterol from the bark of *M. inermis* has been identified as β-sitosterol.

This communication is concerned mainly with the isolation and characterisation of the alkaloids from several species of *Mitragyna*, preliminary to a structural study of this group of alkaloids. The genus *Mitragyna* occurs in the natural order Rubiaceæ, which has provided the cinchona alkaloids and also yohimbine. The isolation of several *Mitragyna* alkaloids has already been reported by other workers. These alkaloids include: mitragynine, probably $C_{22}H_{30}O_4N_2$; mitraphylline (rubradinine), $C_{21}H_{26}O_4N_2$; mitraspecine, $C_{28}H_{36}O_5N_2$ (?); mitraversine, $C_{22}H_{26}O_4N_2$ (?); rhyncophylline, $C_{22}H_{28}O_4N_2$; and rotundifoline, $C_{22}H_{26}O_5N_2$. Their structures have been very little investigated although it is known that only one of the two nitrogen atoms is basic and it has been suggested that the other is in an indole ring. Possibly a carboline system is present (cf. Ing and Raison, *J.*, 1939, 986). In some of these alkaloids two of the oxygen atoms have been shown to be present in a carbomethoxyl group and one in a methoxyl group, but the remaining oxygen atoms have not been characterised.

"Mitrinermine" was isolated from *M. inermis* O. Kuntze by Raymond-Hamet and Millat (*Compt. rend.*, 1934, **199**, 587) and also from *M. stipulosa* O. Kuntze by the same workers (*J. Pharm. Chim.*, 1934, **20**, 577). We have now isolated it from the bark of *M. ciliata* and have established its identity by melting-point comparisons of the free base, its chloroaurate, and its chloroplatinate. It was suggested by Raymond-Hamet (*Compt. rend.*, 1936, **203**, 1383)

that " mitrinermine " might be identical with rhyncophylline, which Kondo, Fukuda, and Tomita (*J. Pharm. Soc. Japan*, 1928, **48**, 321) had isolated from *Ourouparia rhyncophylla* (N. O. Rubiaceæ). This view was supported by toxicity experiments with samples of " mitrinermine " and rhyncophylline (Raymond-Hamet, *Compt. rend. Soc. Biol.*, 1938, **128**, 777) and was accepted by Barger, Dyer, and Sargent (*J. Org. Chem.*, 1939, **4**, 418) who isolated from the leaves of *M. rotundifolia* (Roxb.) O. Kuntze an alkaloid which was indistinguishable from authentic samples of " mitrinermine " and rhyncophylline, and also a new alkaloid, rotundifoline. The identity of " mitrinermine " and rhyncophylline was later challenged by Millat (*Ann. Pharm. franç.*, 1946, **4**, 27) on the ground of supposed differences in elementary composition, colour reactions with Fröhde's reagent, melting point, and specific rotation. The analytical figures of Akamatsu (*Nagasaki Igakkai Zasshi*, 1928, **6**, 333; cf. Millat, *loc. cit.*) do, in fact, suggest $C_{21}H_{26}O_4N_2$ as the formula for rhyncophylline, but those of Kondo (*loc. cit.*) are in agreement with $C_{22}H_{28}O_4N_2$, which is also the formula for " mitrinermine." Moreover, M. Raymond-Hamet has informed us that the colour normally given by rhyncophylline with Fröhde's reagent is due to an impurity. Also, the melting point of mitrinermine is not 203°, as cited by Millat, but 215—216° (Raymond-Hamet and Millat, *loc. cit.*; *Bull. Sci. Pharmacol.*, 1934, **41**, 533), which agrees with that of rhyncophylline. We have carried out mixed melting point determinations with mitrinermine isolated from *M. inermis* and the alkaloid isolated by Barger, Dyer, and Sargent (*loc. cit.*) from *M. rotundifolia* and regarded by them as rhyncophylline. There was no depression, and this applies also to the chloroaurates and chloroplatinates prepared from the alkaloid from each source. The figures for these were in good agreement with those cited by Kondo for the rhyncophylline derivatives. Finally, a careful comparison of the specific rotation of the alkaloid from each source, using light of different wave-lengths, failed to show any differences. It is noteworthy that the specific rotations of this and other *Mitragyna* alkaloids vary very greatly with concentration.

Barger *et al.* (*loc. cit.*) described some degradation experiments with rhyncophylline. We have now obtained evidence that its molecule contains one double bond. Its solution in chloroform decolorises bromine without liberation of hydrogen bromide although it gives no colour with tetranitromethane. Hydrogenation in acetic acid solution did not take place with a palladium catalyst, but with Adams's platinum catalyst there was absorption of one molecular proportion of hydrogen.

From the leaves of *M. rotundifolia* Barger *et al.* (*loc. cit.*) isolated, not only rhyncophylline, but also rotundifoline. We have simplified the separation of these two alkaloids and have also isolated rotundifoline from the leaves of *M. ciliata*, from the bark of which we have obtained rhyncophylline. Although it is unusual for two alkaloids to occur, one in the bark and the other in the leaves of the same species, this is by no means unknown. The co-existence of the two alkaoids in both *M. ciliata* and *M. rotundifolia* suggests a close chemical relationship and it may be that rotundifoline is an oxidation product of rhyncophylline.

By the selenium dehydrogenation of rotundifoline Barger *et al.* (*loc. cit.*) obtained a mixture of bases from which they isolated a picrate, m. p. 134—135°, of composition indicating a base $C_9H_{13}N$. Witkop (*J. Amer. Chem. Soc.*, 1948, **70**, 3713) has shown that this base is not 2 : 4-dimethyl-3-ethylpyridine, the picrate of which has a similar melting point. Koenigs and Bernhart (*Ber.*, 1905, **38**, 3049) give m. p. 139° for the picrate of 3 : 4-diethylpyridine, which has the same composition, and we undertook some preliminary experiments on the synthesis of this base. Recently Karrer and Enslin (*Helv. Chim. Acta*, 1949, **32**, 1390) have described the formation of 3 : 4-diethylpyridine by degradation of corynanthyrine, which is itself formed by selenium dehydrogenation of the alkaloid corynantheine. We were fortunate enough to obtain a sample of Barger's picrate, given to us by Dr. Klyne, and found that its melting point was not depressed by admixture with synthetic 3 : 4-diethylpyridine picrate for a sample of which we are indebted to Professor Karrer. It would seem, therefore, that 3 : 4-diethylpyridine is a product of selenium dehydrogenation of rotundifoline.

With bromine in chloroform solution and with palladium and platinum catalysts in acetic acid, rotundifoline behaved exactly like rhyncophylline. *Dihydrorotundifoline*, obtained by hydrogenation with Adams's platinum catalyst, was isolated and characterised, and *rotundifoline dibromide* was also prepared. Rotundifoline gave a deep yellow colour with tetranitromethane, and also gave colours with Mandelin's and Erdmann's reagents but not with Fröhde's reagent. The similarity of the rotations of rotundifoline and dihydrorotundifoline suggests that the ethylenic bond in the former is unconjugated and this was confirmed by comparison of the absorption spectra, for which we are indebted to Dr. E. Clar who found that the two compounds gave almost identical spectra.

Through the good offices of Dr. W. Klyne, and the kindness of Drs. Dyer and Sargent, we have been able to examine some of the crude phenolic alkaloid extracted by Barger, Dyer, and Sargent (*loc. cit.*) from the leaves of *M. rotundifolia*. This was purified by chromatography on alumina and yielded a new crystalline alkaloid, *mitragynol*, $C_{21}H_{26}O_5N_2$, characterised as its *hydrochloride*. The colour reactions of mitragynol with Erdmann's, Frōhde's, and Mandelin's reagents were similar to those of rhyncophylline and rotundifoline, and ferric chloride in ether gave a red colour. Tetranitromethane gave an orange colour with mitragynol, but there was no other evidence of unsaturation; the compound did not decolorise bromine in chloroform, nor did it absorb hydrogen over Adams's platinum catalyst in acetic acid. Mitragynol appeared to have the character of a phenol, for it was soluble in sodium hydroxide solution, but not in sodium hydrogen carbonate solution, and gave a reddish-brown colour with ethanolic ferric chloride. By treatment with diazomethane mitragynol was converted into methylmitragynol which was insoluble in alkali and gave very little intensification of colour with ethanolic ferric chloride. Methylmitragynol proved to be identical with dihydrorotundifoline.

Differentiation between rotundifoline and dihydrorotundifoline was not easy, as the two compounds had almost the same melting point and there was no depression on mixing them. This applied also to the chloroplatinates and we did not succeed in preparing other crystalline salts. However, Barger's specimen of rotundifoline, isolated from *M. rotundifolia*, absorbed hydrogen equivalent to one double bond and also gave a very dark red colour with ferric chloride in ether. These characteristics were likewise shown by the rotundifoline which we isolated from *M. ciliata*, but not by dihydrorotundifoline or methylmitragynol, which we regard as identical. Moreover, by treatment of the last two substances with bromine under conditions which led to the formation of a dibromide from rotundifoline, brominated products could not be isolated. Rotundifoline gave a permanent flocculent precipitate with antimony pentachloride in chloroform; dihydrorotundifoline and methylmitragynol gave only a temporary haziness with this reagent.

Methoxyl determinations on these compounds gave results for which an explanation cannot yet be given. The determinations were carried out by Pregl's micro-modification of the Zeisel method. Mitragynol gave a value indicating the presence of two methoxyl groups; methylmitragynol, which is apparently obtained by methylation of a phenolic or enolic hydroxyl group also gave a value corresponding with two methoxyl groups. This latter value accords with that quoted by Barger *et al.* (*loc. cit.*), which we have confirmed, for rotundifoline. These authors found no *N*-methyl in rotundifoline. It appears, therefore, that the action of diazomethane on mitragynol methylates an acidic hydroxyl group to give a methoxyl group which is not hydrolysed under the conditions of the Zeisel determination. An example of failure of a methoxy-compound to respond to this analytical treatment had already been encountered by Dr. R. Schoental in these laboratories, and her results are recorded in an appendix to this communication. In her case lack of solubility may be the explanation, but this cannot apply in the cases of rotundifoline and methylmitragynol (dihydrorotundifoline).*

Mitraphylline has been isolated from *M. rubrostipulaceæ* (*Adina rubrostipulata* K. Schumann) by Michiels (Michiels and Leroux, *Bull. Acad. roy. med. Belg.*, 1925, **5**, 403; Michiels, *J. Pharm. Belg.*, 1935, **17**, 1049), by Denis (*Bull. Acad. roy. Belg.*, 1937, **23**, 174) who named it rubradinine, and by Raymond-Hamet (*Bull. Sci. pharmacol.*, 1939, **46**, 327); Michiels (*J. Pharm. Belg.*, 1931, **13**, 719) also isolated it from *M. stipulosa* (*M. macrophylla*). We have examined several samples of *M. rubrostipulaceæ* and have isolated *lævo*-mitraphylline from the leaves and *dextro*-mitraphylline from the bark. Michiels (*J. Pharm. Belg.*, 1931, **13**, 719) has reported " inactive " mitraphylline, and we also have isolated material which showed no optical rotation. Mitraphylline gave a yellow colour with tetranitromethane and formed a *dibromide*. It absorbed one molecule of hydrogen when treated in acetic acid with hydrogen and a palladium catalyst. With Adams's platinum catalyst one molecular proportion of hydrogen was rapidly absorbed, and two further molecular proportions were slowly taken up.

During extractions of the alkaloids from various species of *Mitragyna* we have observed the presence of a triterpene acid in considerable amount. This acid was isolated from the bark of *M. inermis* and from both bark and leaves of *M. ciliata* and *M. rubrostipulaceae*. In every case it has been identified as quinovic acid, long known as a constituent of cinchona bark (natural order Rubiaceæ) (Liebermann, *Ber.*, 1884, **17**, 868) and more recently found in *Zygophyllum coccineum* L., an Egyptian plant of the natural order Zygophyllaceæ (Soliman, *J.*, 1939, 1760).

We have also examined a sample of sterol kindly given to us by M. Raymond-Hamet, who

* See also Nunn and Rapson, *J.*, 1949, 3155.

isolated it from the bark of *M. inermis*. After purification, determinations of the melting points and specific rotations of the sterol, its acetate, and its 3 : 5-dinitrobenzoate indicated that the sterol was β-sitosterol (cf. Ichiba, *Inst. Phys. Chem. Res., Tokyo*, 1935, **28**, 112; Simpson and Williams, *J.*, 1937, 733), and this view was supported by direct comparison with authentic specimens of β-sitosterol and its corresponding derivatives (Cook and Paige, *J.*, 1944, 336).

Experimental.

Rhyncophylline.—A mixture of finely ground *M. inermis* bark (15 kg.) and calcium oxide (5 kg.) was moistened, air-dried at 40°, and then extracted with hot chloroform in a continuous extractor until a sample of extract no longer gave a precipitate with Mayer's reagent. After removal of the chloroform the residual paste was extracted with 3% hydrochloric acid until the residue was free from basic material. The alkaloid (2 g.) was then precipitated by addition of concentrated ammonia solution. Rhyncophylline then crystallised from methanol in small colourless prisms, m. p. 212—213°, not depressed by admixture with specimens supplied by Dr. Klyne, extracted from both *M. inermis* and *M. rotundifolia*. The chloroplatinate formed orange prisms (from methanol) which decomposed at 236° (Kondo *et al.*, *loc. cit.*, give 238° for the decomposition point of the chloroplatinate of rhyncophylline). The chloroaurate melted at 132° and then decomposed at 155° (Kondo gives 134° and 155°, respectively).

Similar extraction of the bark (500 g.) of *M. ciliata* gave crystalline rhyncophylline (0·1 g.), m. p. 211°; its chloroplatinate decomposed at 236—237°, and its chloroaurate at 155—157°, after melting at 133°. Mixed-m. p. determinations showed no depressions.

A specimen of rhyncophylline extracted from *M. rotundifolia* by Barger, Dyer, and Sargent (*loc. cit.*) gave a chloroplatinate which formed orange prisms and decomposed at 235° after darkening (Found : C, 44·6; H, 5·0; Pt, 16·2. Calc. for $C_{44}H_{58}O_8N_4Cl_6Pt$: C, 44·8; H, 5·0; Pt, 16·55%), and a chloroaurate which melted at 132° and then decomposed at 155°. These values also showed no depressions when the specimens were mixed with corresponding compounds of rhyncophylline prepared from *M. inermis*.

Optical Rotations.—The following measurements, made with chloroform solutions at 19° ($l = 1$) serve to confirm the identity of the alkaloid (*I*) from *M. inermis* with that (*R*) isolated by Barger *et al.* (*loc. cit.*) from *M. rotundifolia* :

Sample.	Concn.	Wave-length of source :	[α]. 7000.	6500.	5900.	5500.	5000.
R	5·30		—	—	−6·0°	—	—
R	2·35		—	−12·6°	−14·3	−15·3°	−16·6°
I	2·26		−12·6°	−14·6	−15·7	−17·5	−18·7
R	1·00		−16·0	−18·0	−22·0	−28·0	−36·0
I	1·13		−17·7	−20·4	−23·0	−28·3	−37·2

Rotations were also measured of solutions in 2N-hydrochloric acid at 19° ($l = 1$) as follows (concentrations given as weight of base in 100 c.c. of acid) :

	Concn.	7000.	6500.	5900.	5500.	5250.	5000.
R	1·50	+1·0°	+1·7°	+3·3°	+4·7°	+6·7°	+8·3°
I	1·50	+1·0	+1·7	+2·7	+4·0	+6·0	+8·0

Specific rotations given in the literature for rhyncophylline are $[\alpha]_D^{13}$ −14·7° (Kondo *et al.*) and $[\alpha]_D^{15}$ −14·5° (*c*, 2·5 in chloroform) (Barger *et al.*). For " mitrinermine," Raymond-Hamet and Millat (*J. Pharm. Chim.*, 1934, **20**, 577) give [α] −23·0° (*c*, 1·45), −23·1° (*c*, 1·51), −26·4° (*c*, 1·45), and −26·5° (*c*, 1·19) (all in chloroform).

Rotundifoline.—A mixture of the minced leaves of *M. ciliata* (500 g.) and calcium oxide (100 g.) was moistened, dried, and extracted for 16 hours with hot chloroform. The extracted alkaloid was crystallised from ethanol (yield, 0·8 g.) and had m. p. 233°, not depressed on admixture with an authentic specimen of the rotundifoline of Barger *et al.* (*loc. cit.*), who give m. p. 233—234°. The observation that rotundifoline is sparingly soluble in chlorobenzene whereas rhyncophylline is easily soluble made it possible to effect a ready separation of these two alkaloids from the crude mixture obtained from *M. rotundifolia*. Polarimetric comparison (in chloroform at 21°; $l = 1$) of the alkaloid from *M. ciliata* (*C*) with Barger's rotundifoline (*Ro*) confirmed their identity.

	Concn.	7000.	6500.	5900.	5500.	5250.	5000.	4750.
C	2·10	+85·7°	+98·6°	+123·5°	+145·2°	+163·9°	+184·8°	+217·6°
Ro	2·10	+84·8	+97·6	+122·4	+143·8	+162·4	+184·3	+214·3

[Barger *et al.*(*loc. cit.*) give $[\alpha]_D^{15}$ + 124° (*c*, 2·14 in chloroform)].

The following rotations were observed for solutions of rotundifoline in 2N-hydrochloric acid at 21° (*c*, 2·48 g. per 100 c.c.; $l = 1$) :

7000.	6500.	5900.	5500.	5250.	5000.	4750.
+44·8°	+48·4°	+60·9°	+70·6°	+79·0°	+90·7°	+109·6°

With Mandelin's reagent rotundifoline gave a brown colour which became olive-green on storage; with Fröhde's reagent there was no colour, and with Erdmann's reagent no immediate colour but a light brown colour developed on storage. A methoxyl determination was carried out on the sample of Barger's rotundifoline supplied by Dr. Klyne (Found : OMe, 16·2. Calc. for $C_{22}H_{26}O_5N_2$: 2OMe, 15·6%). The

chloroplatinate of rotundifoline had m. p. 232—234° (decomp.) (Found: C, 43·4; H, 5·1. $C_{44}H_{54}O_{10}N_2Cl_6Pt$ requires C, 43·7; H, 4·6%).

Rotundifoline was not hydrogenated in ethanolic solution with Adams's platinum catalyst or with Raney nickel at 75°/30 atmospheres. Attempted micro-hydrogenation in acetic acid with palladium-black was likewise unsuccessful, but with Adams's platinum catalyst in acetic acid one molecular proportion of hydrogen was absorbed. Repetition on a macro-scale led to the isolation of *dihydrorotundifoline*, which formed colourless needles (from methanol), m. p. 233°, not depressed on admixture with rotundifoline (Found: C, 65·6; H, 7·3. $C_{22}H_{28}O_5N_2$ requires C, 66·0; H, 7·1%). The rotation of dihydrorotundifoline in chloroform at 21° (*c*, 2·41) was as follows:

7000.	6500.	5900.	5500.	5250.	5000.	4750.
+96·7°	+103·7°	+123·7°	+147·7°	+163·5°	+189·6°	+220·3°

The chloroplatinate of dihydrorotundifoline had m. p. 224—226° (from methanol), not depressed on admixture with the chloroplatinate of methylmitragynol described below.

Rotundifoline was treated with bromine in chloroform at room temperature until no more was taken up, and the product was heated under reflux with acetone to reduce perbromide. The resulting *dibromide* was further purified by repeated dissolution in dilute hydrochloric acid and reprecipitation with aqueous ammonia. It formed an amorphous powder, m. p. 215° (Found: C, 47·2; H, 4·8; Br, 28·8. $C_{22}H_{26}O_5N_2Br_2$ requires C, 47·3; H, 4·7; Br, 28·6%).

3 : 4-*Diethylpyridine Picrate*.—A sample of the picrate of the base formed by selenium dehydrogenation of rotundifoline (Barger, Dyer, and Sargent, *loc. cit.*), given to us by Dr. Klyne, had micro-m. p. 131—133°. Its m. p. was not depressed when the salt was mixed with a specimen of 3 : 4-diethylpyridine picrate (micro-m. p., 134—136°) presented by Professor Karrer.

Mitragynol.—The pale brown amorphous phenolic alkaloid (10 g.) from *M. rotundifolia* (Barger *et al.*, *loc. cit.*), for which we are indebted to Dr. Klyne, was dissolved in chloroform (100 c.c.), and its solution passed through a column of alumina (2 × 12 cm.), previously saturated with benzene. The column was eluted with chloroform (150 c.c.). Evaporation of the solvent gave an amorphous mass which crystallised on storage with methanol (yield of colourless crystals, 5·8 g.). *Mitragynol* crystallised from methanol in colourless transparent needles which became opaque on storage. After being dried in a vacuum, it had m. p. *ca.* 130°, depending on the rate of heating (Found: C, 65·3; H, 6·9; N, 7·1; OMe, 15·45, 14·8. $C_{21}H_{26}O_5N_2$ requires C, 65·25; H, 6·8; N, 7·25; 2OMe, 16·0%). Specific rotations at different concentrations and wave-lengths were:

Concn.	7000.	6500.	5900.	5500.	5250.	5000.
4·24	+2·8°	+1·4°	+0·5°	−0·5°	−0·7°	−2·1°
2·12	−2·8	−3·8	−4·7	−5·2	−6·6	−8·0
1·06	−5·7	−7·6	−8·7	−10·4	−12·3	−13·2

The *hydrochloride*, prepared in aqueous solution and recrystallised from water, formed colourless needles, m. p. 212—216° (Found: C, 59·6; H, 6·2; N, 6·8. $C_{21}H_{27}O_5N_2Cl$ requires C, 59·6; H, 6·4; N, 6·6%). The analytical specimen was dried at 100° *in vacuo*. Specific rotations in aqueous solution at 21° (*c*, 3·75; *l* = 1) were:

7000.	6500.	5900.	5500.	5250.	5000.	4750.
−30·9°	−34·9°	−42·9°	−51·4°	−57·1°	−64·3°	−71·4°

With Mandelin's reagent mitragynol gave a transient brown colour, changing to olive-green; with Fröhde's reagent there was no colour and with Erdmann's reagent a very faint brown colour on storage. Tetranitromethane gave an orange colour. Mitragynol gave no sparingly soluble chloroplatinate and did not absorb hydrogen in acetic acid over Adams's platinum catalyst.

For methylation, a solution of mitragynol (0·6 g.) in ether and methanol was set aside overnight with excess of ethereal diazomethane. The residue obtained by evaporation was recrystallised from methanol, giving colourless needles (0·46 g.), m. p. 232—233°, not depressed on admixture with dihydrorotundifoline (above) (Found: C, 65·8; H, 6·9; OMe, 15·4. Calc. for $C_{22}H_{28}O_5N_2$: C, 66·0; H, 7·1; OMe, 15·6%). Identity was confirmed by preparation of the *chloroplatinate*, which crystallised from ethanol in orange-yellow prisms, m. p. 224—226° (Found: C, 43·6; H, 5·1; Pt, 15·7. $C_{44}H_{58}O_{10}N_4Cl_6Pt$ requires C, 43·6; H, 4·8; Pt, 16·1%). The following rotations (in chloroform at 21°; *l* = 1) were observed with the dihydrorotundifoline (methylmitragynol) prepared in this way:

Concn.	7000.	6500.	5900.	5500.	5250.	5000.	4750.
3·28	+86·6°	+102·1°	+122·6°	+144·5°	+163·4°	+186·9°	+218·3°
1·64	+92·7	+109·7	+126·3	+151·2	+166·9	+194·5	+223·8
0·822	+88·0	+109·7	+128·0	+154·9	+178·0	+202·4	+225·5

Mitraphylline.—(*a*) Finely ground bark of *M. rubrostipulaceæ* (500 g.) was mixed with calcium oxide (125 g.), moistened, air-dried, and extracted for 16 hours with hot chloroform. The mitraphylline recovered from the extract formed long colourless needles, m. p. 262° (from methanol), and gave a picrate, m. p. 165° (Raymond-Hamet and Millat, *Bull. Sci. Pharmacol.*, 1935, **42**, 602, give m. p. varying between 258° and 267° for the alkaloid, and Denis, *loc. cit.*, gives m. p. 166° for the picrate). Specific rotations of the base in chloroform at 21° (*l* = 2) were:

Concn.	7000.	6500.	5900.	5500.	5250.	5000.	4750
1·96	+4·3°	+5·4°	+7·1°	+9·2°	+11·7°	+14·8°	+19·1°
0·98	+2·5	+3·5	+4·6	+5·6	+7·7	+10·2	+13·3

In 2N-hydrochloric acid at 21° ($l = 1$; concentrations in g. of base per 100 c.c.) the rotations were :

Concn.	7000.	6500.	5900.	5500.	5250.	5000.	4750.
2·70	+18·6°	+22·6°	+26·7°	+30·4°	+34·1°	+37·8°	+41·9°
1·35	+14·8	+17·8	+21·5	+28·9	+32·6	+36·3	+40·8

(*b*) Finely ground leaves of *M. rubrostipulaceæ* (500 g.), extracted similarly, gave the same alkaloid (2·5 g.), m. p. 261° alone or mixed with the sample described in the previous paragraph or with mitraphylline kindly given us by M. Raymond-Hamet. The picrate had m. p. 165°, not depressed by the picrate of the alkaloid from the bark A sample of leaves supplied about 6 months later gave only one-tenth of the amount of alkaloid for the same weight of leaves; this was lævorotatory, having $[\alpha]_D^{20}$ −7·4° (*c*, 1·20 in chloroform) (Found : C, 68·3; H, 7·0; N, 7·3; OMe, 8·5. Calc. for $C_{21}H_{26}O_4N_2$: C, 68·0; H, 7·0; N, 7·6; OMe, 8·4%). For mitraphylline in chloroform, Raymond-Hamet and Millat (*loc. cit.*) give $[\alpha]_D$ −7·7° (*c*, 3·9) and −5·8° (*c*, 1·2), Raymond-Hamet (*Bull. Sci. Pharmacol.*, 1939, **46**, 327) gives $[\alpha]_D^{15}$ −9·8° (*c*, 0·51) and Denis (*loc. cit.*) gives $[\alpha]_D$ −22·27° (*c*, 4·8), whereas Michiels and Delvaux (*J. Pharm. Belg.*, 1931, **13**, 719) describes mitraphylline as having zero rotation. More recently we also have extracted the alkaloid from about a hundredweight of bark and leaves of *M. rubrostipulaceæ* and have isolated optically inactive mitraphylline.

Microhydrogenation of mitraphylline in acetic acid over Adams's platinum catalyst led to absorption of 3 moles of hydrogen. The first molecule was taken up in 18 minutes, and the other two each required 40 minutes. Microhydrogenation over palladium-black led to absorption of one mole of hydrogen.

Treatment of mitraphylline with bromine in chloroform at room temperature gave an amorphous product which was heated under reflux with acetone to decompose perbromide and then purified by repeated solution in dilute hydrochloric acid and reprecipitation with aqueous ammonia. The dibromide, an amorphous powder, m. p. 195—198°, was converted into its *chloroplatinate* which formed small yellow leaflets (from methanol) and decomposed at 190° (Found : C, 34·4; H, 3·95; Pt, 12·5. $C_{42}H_{54}O_8N_4Cl_6Br_2Pt$ requires C, 34·3; H, 3·7; Pt, 13·3%).

Quinovic Acid.—The *M. inermis* bark from which the alkaloid had been extracted (above) was kept for 24 hours in 10% sodium carbonate solution (20 galls.), and then percolated with a further equal volume. The bark was pressed manually, and the combined extracts were treated with " Supercel " and then acidified with 15% hydrochloric acid. The filtered precipitate was re-dissolved in alkali and reprecipitated after filtration from the " Supercel." The crude acid was purified by repeated crystallisation from ethanol, with charcoal. The yield of almost pure acid was 50 g.

The pure acid formed colourless crystals (Found : C, 73·8, 74·1; H, 9·3, 9·4%; equiv., by titration, 240, 248, 249. Calc. for $C_{30}H_{46}O_5$: C, 74·0; H, 9·5%; equiv., 243), m. p. 298° (alone or mixed with an authentic specimen of quinovic acid kindly presented by Professor L. Ruzicka), $[\alpha]_D^{20}$ +99° (*c*, 2·48 in pyridine). For the potassium salt (concn. expressed as g. of acid in potassium carbonate solution), $[\alpha]_D^{19}$ = +89° (*c*, 0·98) (lit. : m. p. of quinovic acid, 298°; $[\alpha]_D^{16}$ of potassium salt, +87°).

The methyl ester, prepared with excess of ethereal diazomethane, formed colourless crystals (from light petroleum), m. p. 172—173°, not depressed in admixture with an authentic specimen of methyl quinovate. It had $[\alpha]_D^{19}$ +117·4° (*c*, 2·12 in chloroform) (lit. : m. p. 173—174°; $[\alpha]_D^{19}$ +115·2°, +116·8°, in chloroform).

The acetyl derivative, prepared by heating the acid with excess of acetic anhydride under reflux, crystallised from methanol in colourless needles, m. p. 281—282° (lit., 284°) (Found : C, 73·0, 72·8; H, 9·05, 9·2. Calc. for $C_{32}H_{48}O_6$: C, 72·6; H, 9·15%).

Quinovic acid was also isolated from both bark and leaves of *M. ciliata* and *M. rubrostipulaceæ*. The following constants were given by the various specimens, and in all cases mixed m. p.s with authentic samples were carried out. The specific rotations are for the potassium salt, in water.

Source :	*M. inermis* bark.	*M. ciliata* bark.	*M. ciliata* leaves.	*M. rubrostipulaceæ* bark.	*M. rubrostipulaceæ* leaves.
M. p. of acid	298°	300°	298°	303°	301°
$[\alpha]_D^{20}$	+86	+87	+87	+43, +44	+86
M. p. of acetyl derivative	281—282°	280—281°	283°	284°	281—281·5°
M. p. of methyl ester	172—173°	172—173°	171—173°	171—173°	172—174°

β-Sitosterol from M. inermis.—A sample of a sterol obtained from benzene extracts of *M. inermis* bark, after removal of rhyncophylline, was presented to us by M. Raymond-Hamet. Repeated recrystallisation from methanol gave crystals of m. p. 134—135° and $[\alpha]_D^{20}$ −35·6° (*c*, 1·54 in chloroform). This sterol, which was unsaturated, was converted into its acetate by acetic anhydride in pyridine at room temperature, and into its 3 : 5-dinitrobenzoate by 3 : 5-dinitrobenzoyl chloride in boiling pyridine. The m. p.s of the sterol and its two derivatives were not depressed on admixture with β-sitosterol and the corresponding derivatives, isolated by Cook and Paige (*loc. cit.*) from *Calycanthus floridus*, and the satisfactory agreement between constants, given in the following table, confirms the identification of the sterol from *M. inermis* as β-sitosterol.

	Sterol from *M. inermis*.	β-Sitosterol.
M. p. of sterol	134—135°	137·5—138·5° [1]
$[\alpha]_D$ of sterol	−35·6	−34·0 [1]
M. p. of acetate	128°	126·5—127·5° [1]
$[\alpha]_D$ of acetate	−38·5 [2]	−34·7 [3]
M. p. of 3 : 5-dinitrobenzoate	203—204°	208—209° [1]
$[\alpha]_D$ of 3 : 5-dinitrobenzoate	−20·7 [4]	−21·7 [3]

[1] Cook and Paige's values (*loc. cit.*), *q.v.* for figures given by earlier workers. [2] *c*, 1·20 in chloroform at 21°. [3] Simpson and Williams, *J.*, 1937, 733. [4] *c*, 1·20 in chloroform at 20°.

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Appendix. By Miss R. Schoental.

In connection with a study of the metabolic oxidation products of some polycyclic aromatic hydrocarbons, specimens have been prepared of the dimethyl ethers of the anthraquinols formed by alkaline reduction of three anthraquinone derivatives. Unexpected difficulty was encountered in the analysis of 9 : 10-dimethoxy-1 : 2 : 5 : 6-dibenzanthracene. Three attempts (by Mr. J. M. L. Cameron) to estimate the methoxyl content by Pregl's micro-modification of the Zeisel method gave a zero value, although the compound should contain two methoxyl groups. There can be little doubt that the structure is correctly assigned, for it is supported by the method of preparation and the elementary analysis of the compound and its picrate; moreover, the ultra-violet absorption spectrum of the dimethoxy-compound resembles very closely that of 1 : 2 : 5 : 6-dibenzanthracene (Mayneord and Roe, *Proc. Roy. Soc.*, 1935, *A*, **152**, 320), with a shift towards the region of longer wave-length, which amounted to 100 A. for the longest band. This failure to hydrolyse the dimethyl ether is possibly caused by steric hindrance, but the compound is very sparingly soluble and this may be a contributory factor. Two other compounds of similar structure gave somewhat low values for the methoxyl content, which however clearly indicated the presence of two methoxyl groups in each case.

For preparation of these dimethoxy-compounds, the appropriate quinone was heated with zinc dust, aqueous sodium hydroxide, and methyl sulphate, the alkali and methyl sulphate being added alternately in small portions until the alkaline solution was almost completely decolorised. The product was extracted with benzene and the strongly fluorescent benzene solution was purified by chromatography on alumina. The concentrated benzene eluates were treated with light petroleum.

9 : 10-*Dimethoxy*-1 : 2-*benzanthracene* formed colourless rhombic plates, m. p. 137—138° (Found : C, 83·35; H, 5·7; OMe, 19·3. $C_{20}H_{16}O_2$ requires C, 83·3; H, 5·6; OMe, 21·5%) (cf. Berenblum and Schoental, *Cancer Res.*, 1943, **3**, 686). 9 : 10-Dimethoxy-1 : 2 : 7 : 8-dibenzanthracene could not be obtained crystalline, but formed a *s-trinitrobenzene* complex which crystallised from ethanol in orange-yellow needles, m. p. 157—158° (Found : C, 65·35; H, 3·85; N, 7·7; OMe, 9·9. $C_{30}H_{21}O_8N_3$ requires C, 65·3; H, 3·8; N, 7·6; OMe, 11·2%). 9 : 10-*Dimethoxy*-1 : 2 : 5 : 6-*dibenzanthracene* formed colourless rhombic crystals, m. p. 234—235·5° (Found : C, 85·3; H, 5·5. $C_{24}H_{18}O_2$ requires C, 85·2; H, 5·4%); its *dipicrate* crystallised from benzene in deep-red needles, m. p. 183—184° (decomp.) (Found : C, 54·2; H, 2·9; N, 10·5. $C_{36}H_{24}O_{16}N_6$ requires C, 54·3; H, 3·0; N, 10·55%).

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