210. *The Alkaloids of* Mitragyne speciosa. *Part I. Mitragynine.*

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This paper describes preliminary work on the structure of mitragynine, the chief alkaloid of the leaves of *M. speciosa*. A second alkaloid has been detected, but not characterised because of the amorphous nature of its salts.

Mitragynine has been given the formula $C_{22}H_{30}O_4N_2$ (or possibly $C_{22}H_{32}O_4N_2$); it is the methyl ester of a monocarboxylic acid, which contains in addition two methoxyl groups but no methylimido-group. It behaves as a monoacidic base. On hydrolysis in alcoholic (ROH) alkali, it appears to form a relatively stable intermediate substance, $C_{22}H_{30}O_4N_2$,ROH, which by the further action of alkali yields the acid $C_{21}H_{28}O_4N_2$.

Zinc dust distillation produces a base, $C_{14}H_{14}ON_2$, which may be an *N*-methylmethoxyharman, together with unidentified substances which give indole colour reactions.

The genus *Mitragyne* belongs to the important natural order of *Rubiaceæ*, but has attracted little serious attention from either chemists or pharmacologists. Several alkaloids have been isolated from various members of the species, *viz.*, mitraversine (Field, J., 1921, **119**, 887; Raymond-Hamet and Millat, *J. Pharm. Chim.*, 1937, **25**, 391), mitragynine (Field, *loc. cit.*), mitraspecine (Denis, *Bull. Acad. roy. Belg.*, 1938, **24**, 653), mitraphylline (Michiels, *J. Pharm. Belg.*, 1931, **13**, 159, 719), and mitrinermine (Raymond-Hamet and Millat, *Compt. rend.*, 1934, **199**, 587; *J. Pharm. Chim.*, 1934, **126**, 577), but only the amorphous mitragynine (from *M. speciosa*) has been examined in detail (Field, *loc. cit.*). This alkaloid was given the formula $C_{22}H_{31}O_5N$ and was said to contain two carbomethoxy-groups, and various colour reactions indicated the presence of an indole nucleus.

For the present work the dried leaves of *M. speciosa* (" Kratom " of the natives) were obtained from Siam through the courtesy of the Director General of the Government Laboratory, Bangkok. A 70% aqueous-alcoholic extract of the leaves on evaporation left an aqueous layer and a resinous solid, from both of which two picrates were obtained; one was mitragynine picrate (m. p. *ca.* 220°), and the other melted at 123—127°. Choline was the only substance isolated from the mother-liquor of the former.

Mitragynine has been found to have the formula $C_{22}H_{30}O_4N_2$ (or possibly $C_{22}H_{32}O_4N_2$). No crystalline derivative has been obtained from the amorphous picrate of m. p. 123—127°, but preliminary examination shows that it may contain one or more alkaloids isomeric with mitragynine. The four oxygen atoms of the latter are accounted for as two methoxyl

and one carbomethoxy-group. It is a monoacidic base, yields a *monomethiodide*, and cannot be acetylated, and hence we regard the second nitrogen atom as a non-basic indole nitrogen. The base does not absorb hydrogen in presence of palladium or platinum, and the only recognisable permanganate oxidation products were oxalic and acetic acids.

In view of Field's work the alkaline hydrolysis was studied. Heating with methyl-alcoholic potassium hydroxide gave two products (cf. Field). The ether-insoluble product appears to be a monocarboxylic acid, $C_{21}H_{28}O_4N_2$, containing two methoxyl groups, characterised as the *picrate*, and the ether-soluble basic product a tetramethoxy-compound, $C_{23}H_{34}O_5N_2$ (also characterised as the *picrate*). The latter substance corresponds to the addition of a molecule of methyl alcohol to mitragynine, and further heating with alcoholic potassium hydroxide converts it into the ether-insoluble compound. This does not regenerate mitragynine on methylation.

Ethyl-alcoholic potassium hydroxide gives the same amphoteric product, but the ether-soluble basic substance simultaneously formed is not identical with that obtained in the methyl alcohol hydrolyses.

Selenium dehydrogenation gave no crystalline product; demethylation was indicated by the evolution of dimethyl diselenide (cf. Sharp, J., 1938, 1353). Zinc dust distillation according to Winterstein and Walter (*Helv. Chim. Acta*, 1927, **10**, 577) gave a non-basic fraction, probably containing indole derivatives, and a basic fraction, which gave no indole reactions. It contained a base, $C_{14}H_{14}ON_2$, with one methoxyl and one methyl-imido-group, and a reactive methylene group as shown by the formation of a p-*nitrobenzylidene* derivative. The marked fluorescence of this base in dilute acid solution suggests the possibility of its having a carboline structure; it is, however, not identical with either *pyr-* or *ind-N*-methylharmine, specimens of which were kindly supplied by Sir Robert Robinson, although the superficial similarity is marked.

Experimental.

Extraction of the Alkaloids of M. speciosa.—The following method is an improvement on that of Field (*loc. cit.*). Messrs. T. and H. Smith of Edinburgh kindly prepared a concentrated 70% aqueous-alcoholic extract of the leaves of *M. speciosa* in the form of a dark-coloured aqueous fraction (*A*) and a black resinous solid (*B*).

(*A*) The aqueous fraction (250 g.) was diluted with water (500 c.c.) and acidified to Congo-red with acetic acid, and the precipitated material extracted with ether. The filtered aqueous layer was basified (10% sodium carbonate solution) and again extracted with ether, the aqueous liquid being preserved (*X*). The washed and dried (sodium sulphate) extract left on evaporation a voluminous amorphous residue (*ca.* 1·4 g.), which was dissolved in methyl alcohol (10—15 c.c.) and added to a saturated solution of picric acid in methyl alcohol (15 c.c.). The precipitated mitragynine picrate (0·7 g.) was almost pure (m. p. 215—220°). The mother-liquor was concentrated, diluted with water after removal of any further mitragynine picrate, basified, and extracted with ether. The extract, in 10% acetic acid, gave on addition of picric acid a yellow amorphous picrate (0·3 g.), m. p. 123—127°, which required the addition of sodium chloride for its complete coagulation. 5·3 Kg. of (*A*) yielded 15 g. of mitragynine picrate and 8 g. of the amorphous picrate.

The liquid (*X*), after basic lead acetate treatment, gave a phosphotungstate from which only choline, identified as its mercurichloride and chloroaurate, was isolated.

(*B*) The resinous fraction (500 g.) was dissolved in hot 96% alcohol (400 c.c.), glacial acetic acid (250 c.c.) added, and the liquid gradually added to cold water (4·5 l.) with vigorous stirring. After 12 hours, this was filtered, and the chlorophyll washed with a little water. The filtrate was basified with concentrated aqueous ammonia and shaken with ether. Evaporation of the dried (sodium sulphate) extract left a dark-coloured amorphous mass (*ca.* 40 g.), which was dissolved in the minimum quantity of methyl alcohol and added to saturated methyl-alcoholic picric acid (300 c.c.). The separated orange crystals of mitragynine picrate (*ca.* 40 g.) had m. p. *ca.* 215°. The mother-liquor was concentrated to 125 c.c. and added to aqueous ammonia (15 c.c. of concentrated aqueous ammonia, 750 c.c. of water), and the separated solid dissolved in ether and washed with dilute aqueous ammonia and with water. The residue (7·5 g.) left on evaporation was extracted with 2½% acetic acid (100 c.c.), and the filtered solution added to saturated aqueous picric acid (500 c.c.) together with sodium chloride; the

yellow amorphous picrate obtained (9 g.) had m. p. 123—127°. From 9·5 kg. of (*B*) were obtained 716 g. of mitragynine picrate and 180 g. of the amorphous picrate.

Mitragynine picrate. The crude picrate (40 g.) was dissolved in boiling absolute methyl alcohol (3600 c.c.), solvent distilled off until crystallisation began in the boiling liquid (*ca.* 1500 c.c.), and this left to cool. Highly pure mitragynine picrate, m. p. 217—223° (decomp.) (depending on the rate of heating), was obtained in 82% yield, calculated on the crude picrate, and concentration of the filtrate gave another fraction (6%) only slightly less pure [Found for a specimen crystallised several times from acetic acid and twice from absolute methyl alcohol and dried at 80° in a vacuum: C, 54·4, 54·2, 54·0; H, 5·3, 5·3, 5·5; N, 11·3, 10·8, 11·3; OMe, 13·7, 14·1 *; $C_6H_3O_7N_3$ (by nitron), 35·6. Calc. for $C_{22}H_{30}O_4N_2,C_6H_3O_7N_3$: C, 54·6; H, 5·4; N, 11·4; 3OMe, 15·1; $C_6H_3O_7N_3$, 37·2%. Calc. for $C_{22}H_{32}O_4N_2,C_6H_3O_7N_3$: C, 54·5; H, 5·7; N, 11·3; 3OMe, 15·1; $C_6H_3O_7N_3$, 37·1%. Calc. for $C_{22}H_{31}O_5N,C_6H_3O_7N_3$ (Field's formula): C, 54·3; H, 5·5; N, 9·1; 3OMe, 15·0; $C_6H_3O_7N_3$, 37·2%]. No methylimido-group is present [Found: OMe + NMe (expressed as OMe), 14·0%].

Mitragynine. This was prepared by adding a hot saturated acetone solution of the picrate to an excess of dilute aqueous ammonia and extracting the liberated base with ether. The washed and dried extract was strongly fluorescent and left on evaporation a pale brown, amorphous mass, very soluble in most organic solvents, except petrol, to give fluorescent solutions. It was insoluble in water and melted between 105° and 115°. An electrometric titration revealed the presence of only one point of inflexion (Found: equiv., 399. Calc. for $C_{22}H_{30}O_4N_2$: equiv., 386·5).

Mitragynine acetate. A mixture of the base (2 g.) and acetic anhydride (20 c.c.) was warmed gently for ½ hour and left over-night. The silky needles formed, after being washed with a little acetic anhydride and with much petrol, were practically colourless and had m. p. (rapid heating) 175—176° (decomp.). They soon darkened in light and were fairly readily soluble in ether (Found: C, 64·8, 64·8; H, 7·35, 7·2; N, 6·25; OMe, 17·5. Calc. for $C_{22}H_{30}O_4N_2,C_2H_4O_2$: C, 64·6; H, 7·7; N, 6·3; 3OMe, 20·8%). The acetate can be recrystallised from acetic anhydride at *ca.* 80°, but heating beyond this point gives a less pure product. The same salt, prepared from its components in dry ether (Field), separated from ether–acetic acid in woolly needles, m. p. 175—180° (depending on the rate of heating). Field gave m. p. 142°. The two specimens were shown by mixed m. p. to be identical.

Mitragynine cinnamate, prepared from the base and cinnamic acid in ether, was very soluble in alcohol. It separated from hot acetone or methyl ethyl ketone in felted needles, white when freshly precipitated, but rapidly darkening on exposure to light. Crystallised twice from the latter solvent, the pure salt had m. p. 155° (decomp.; darkening from 135°) (Found: C, 68·4; H, 7·2; N, 5·0, 5·1; OMe, 15·8. $C_{22}H_{30}O_4N_2,C_9H_8O_2$ requires C, 69·7; H, 7·2; N, 5·2; 3OMe, 17·4%. Field's formula, $C_{22}H_{31}O_5N,C_9H_8O_2$, requires N, 2·6%).

Mitragynine hydrogen fumarate. A solution of mitragynine in dry ether was treated with fumaric acid (1 mol.) dissolved in the minimum quantity of absolute alcohol; the *salt* settled rapidly as a buff-coloured sandy powder. Twice crystallised from boiling methyl ethyl ketone, it formed a cream-coloured felted mass of needles, decomp. between 190° and 200° [Found: N, 5·6, 5·8. $C_{22}H_{30}O_4N_2,C_4H_4O_4$ requires N, 5·6%. $C_{22}H_{31}O_5N,C_4H_4O_4$ (Field) requires N, 2·8%].

The corresponding salt with maleic acid was very soluble in alcohol and methyl ethyl ketone, and could not be obtained crystalline.

Mitragynine trinitrobenzene compound. Precipitated from hot methyl-alcoholic solutions of equimolecular weights of the constituents, this formed dark red, stout needles, m. p. 146° (rapid decomp.), unchanged after two further crystallisations (Found: C, 57·6; H, 5·5; N, 12·2. $C_{22}H_{30}O_4N_2,C_6H_3O_6N_3$ requires C, 56·1; H, 5·55; N, 11·7%).

Mitragynine monomethiodide. The base (from 2 g. of the picrate) in acetone (50 c.c.) was refluxed with methyl iodide (4 c.c.) for 6 hours. After removal of most of the solvent, the *salt* was precipitated by dry ether as a buff-coloured powder (0·8 g.), very soluble in alcohol and fairly readily soluble in water. For analysis, it was heated on the water-bath with water (charcoal) and obtained as a cream-coloured amorphous powder, m. p. 211·5° (slight darkening from 180°), by addition of potassium iodide to the filtered solution (Found: C, 52·2; H, 6·5; N, 5·1; I, 23·95. $C_{23}H_{33}O_4N_2I$ requires C, 52·3; H, 6·3; N, 5·3; I, 24·0%).

* It is characteristic of mitragynine and its near derivatives that slightly low values for methoxyl determinations are always found. This was sometimes partly corrected by heating with hydriodic acid for longer periods.

The methopicrate is gelatinous and only precipitated by the addition of an electrolyte.

Alkaline Hydrolysis of Mitragynine.—(1) *Methyl-alcoholic potassium hydroxide.* After treatment of mitragynine with 4N-methyl-alcoholic potassium hydroxide for 24 hours at room temperature, there was little evidence of the formation of any acidic product. Refluxing on the water-bath for ½ hour, followed by 24 hours at room temperature, gave two products. After reaction, the alkaline liquid was exactly neutralised with the predetermined quantity of alcoholic hydrogen chloride, filtered from potassium chloride, and evaporated to dryness. The residue was partitioned between ether and dilute aqueous ammonia, the major product being soluble in ether and basic and giving a scarlet *picrate* on treatment of its acid solution with picric acid. This separated from absolute methyl alcohol as an amorphous orange-red powder, m. p. 135—136°, slightly soluble in hot water. It contained four methoxyl groups, gave the same colour reactions as mitragynine, and regenerated the base after refluxing with alcoholic hydrogen chloride [Found: C, 53·7; H, 5·7; N, 10·75, 10·9; OMe, 16·9 (micro), 17·1, 17·2 (semi-micro), 18·0 (semi-micro; heated for twice the standard time *). $C_{23}H_{34}O_5N_2,C_6H_3O_7N_3$ requires C, 53·8; H, 5·8; N, 10·8; 4OMe, 19·1%. Another independent preparation contained: C, 53·9; H, 5·7; N, 10·4, 10·3; OMe, 17·1%].

The second product was only very slightly soluble in ether and was amphoteric. Evaporation of its ammoniacal solution gave, beside dark-coloured impurities, a glassy residue, which formed a picrate soluble in methyl alcohol, but separating from hot water as an amorphous orange-brown powder, m. p. 155° (Found: OMe, 7·5%).

In another experiment, mitragynine was treated with 2N-potassium hydroxide in methyl alcohol for 3 hours on the water-bath. After neutralisation and evaporation of most of the solvent, the amphoteric substance was precipitated by dry ether, and treated in ethereal suspension with diazomethane. This gave an ether-soluble base which was not mitragynine, since it formed an amorphous orange-yellow picrate, soluble in methyl alcohol but separating from hot water with m. p. 122—123°. The ethereal filtrate from the precipitated amphoteric substance, presumably containing the compound $C_{23}H_{34}O_5N_2$, was evaporated, and the residue treated with 4N-potassium hydroxide in methyl alcohol on the water-bath for a further 3 hours. On working up by partition between ether and dilute aqueous ammonia, a small basic portion (orange-red picrate from methyl alcohol, m. p. 132—134°) was found, but the majority of the product was amphoteric. The ammoniacal solution was saturated with carbon dioxide, filtered, just acidified with dilute acetic acid, and again filtered, each operation removing coloured impurities. The ultimate filtrate gave a *picrate* which was soluble in methyl alcohol, but separated from hot water as a hygroscopic amorphous orange-brown powder, m. p. 157°, apparently identical with the picrate of the amphoteric compound previously isolated (Found: C, 54·4; H, 5·5; N, 11·3, 11·5; OMe, 7·7. $C_{21}H_{28}O_4N_2,C_6H_3O_7N_3$ requires C, 53·9; H, 5·4; N, 11·65; 2OMe, 10·3%).

(2) *Ethyl-alcoholic potassium hydroxide.* After being refluxed for 2 hours and then left for 12 hours at room temperature with 2N-potassium hydroxide, mitragynine again yielded two products. The amphoteric portion formed a picrate, m. p. 155°, apparently identical with that obtained in the previous hydrolyses (Found: OMe, 8·6%); the acid obtained by evaporation of its ammoniacal solution, on treatment with diazomethane, yielded a basic product which formed an amorphous, pale yellow picrate, soluble in methyl alcohol but separating from hot water with m. p. 124—125° (Found: OMe, 12·0%). The ether-soluble product formed a pale yellow picrate, very soluble in cold absolute methyl alcohol, and quite unlike its analogue obtained in the hydrolyses in methyl alcohol. It separated from hot water with m. p. 133—134°, and formed a *methiodide*, an amorphous cream-coloured powder, m. p. 145—146° (Found: OMe, 18·8. $C_{22}H_{30}O_4N_2,CH_3I,C_2H_6O$ requires 4OMe, 21·6%).

Permanganate Oxidation of Mitragynine.—Complete oxidation of the alkaloid in aqueous acetone yielded only acetic and oxalic acids, and partial oxidation of the acid $C_{21}H_{28}O_4N_2$ in alkaline solution gave, beside acetic acid, only an amorphous intractable powder.

Zinc Dust Distillation of Mitragynine (cf. Winterstein and Walter, *loc. cit.*).—The alkaloid (5·0 g.) was heated in amounts of 0·2 g. at a time with ten times its weight of zinc dust in small glass tubes so constructed as to allow any high-boiling products to pass immediately into an air-cooled U-tube whilst difficultly condensable vapours were trapped in a tube packed with glass wool. The tubes were heated to dull red heat during 3—4 minutes. The distillates were dissolved in ether and basic substances were removed with dilute hydrochloric acid, liberated with ammonia, and again dissolved in ether. The residue obtained from the ethereal solution

* For the semi-micro methoxyl determinations we are indebted to Dr. R. S. Cahn.

after the acid extraction gave a red pinewood reaction and yielded 0·8 g. of a red-brown oil with an indole-like smell. The basic products (0·5 g.) gave no indole colour reactions, and were distilled at 0·6—0·4 mm.; at a bath temperature of 230° a bright red oil distilled. This yielded a dirty yellow *picrate* (160 mg.) from ether, which, twice crystallised from methyl ethyl ketone, formed bright yellow clusters of needles (20 mg.), m. p. 255—265° (decomp.) (Found : C, 53·3; H, 3·7; N, 15·4; OMe, 6·6; NMe, 4·9. $C_{14}H_{14}ON_2,C_6H_3O_7N_3$ requires C, 52·8; H, 3·8; N, 15·4; 1OMe, 6·8; 1NMe, 6·4%). The base gave a bluish fluorescence in dilute acid solution.

The procedure was repeated with 30 g. of mitragynine, and the basic fraction of the distillate converted directly into a picrate (3·0 g.). Crystallised from the minimum quantity of boiling methyl ethyl ketone (125 c.c.), this yielded yellow crystals (264 mg.), which after two further crystallisations from the same solvent formed yellow felted masses of needles (162 mg.), m. p. 263—264° (decomp.) (Found : C, 53·4; H, 3·7; N, 15·8; OMe, 7·2; NMe, 4·5%. Again the formula $C_{14}H_{14}ON_2$ provides the best interpretation of the analytical figures). This picrate had mixed m. p. with *pyr-N*-methylharmine picrate (harmine methopicrate; m. p. 263°), 240—245°; and with *ind-N*-methylharmine picrate (m. p. 250°), 225°.

A boiling concentrated acetone solution of the picrate (104 mg.) was added to dilute aqueous ammonia, and the mixture exhaustively extracted with ether. The washed and dried extract on evaporation gave a solid residue, which yielded, after two sublimations at 110—115°/0·02 mm., practically colourless bushy needles (40 mg.), m. p. 115—120° (softening at 109°), mixed m. p. with *ind-N*-methylharmine (m. p. 120—122°), 80—95° (Found : C, 74·2; H, 6·5; N, 12·25. $C_{14}H_{14}ON_2$ requires C, 74·3; H, 6·2; N, 12·4%). The *base* gave a pale blue fluorescence in dilute acid solution, distinct from the blue-violet of the two authentic harmine bases; on the other hand, in benzene solution, the unknown base and *ind-N*-methylharmine gave, when viewed in ultra-violet light, the same brilliant blue-violet fluorescence.

The base (10 mg.) was heated with five times its weight of *p*-nitrobenzaldehyde at 250—255° for a few minutes; the cooled mass was boiled with benzene, shaken with 3 drops of dilute hydrochloric acid (1 : 1), and the orange-brown hydrochloride washed with benzene. The liberated *base* crystallised from aqueous alcohol in stout golden-yellow needles (8 mg.), m. p. 255° (Found : C, 70·4; H, 5·5. $C_{21}H_{17}O_3N_3$ requires C, 70·2; H, 4·8%). The *p*-nitrobenzylidene derivative prepared from *ind-N*-methylharmine in the same way had m. p. 238°, mixed m. p. with the above specimen, 215° (Found : C, 69·3; H, 4·8%). It formed stout brick-red needles.

The Amorphous Picrate of m. p. 123—127° (p. 987).—This would not crystallise from any solvent, but some purification was achieved by extraction with hot water. The picrate (1 g.) was dissolved in a little methyl alcohol and precipitated by water. The well-washed precipitate, while still wet, was digested with cold water (250 c.c.) and heated on the water-bath with constant stirring; the solution was filtered, and left to cool after addition of a little sodium chloride. Twice repeated, this procedure yielded an amorphous powder, m. p. 134—136°, but the analysis was not capable of simple interpretation. The portion insoluble in water was crystallised three times from methyl alcohol and identified as mitragynine picrate. The base obtained from the above picrate gave the same colour reactions as mitragynine, and was converted into its perchlorate, acetate, picrolonate, and flavianate, but none could be obtained crystalline. Many other salts were precipitated from aqueous or ethereal solution in a gelatinous condition.

The base was extracted (Soxhlet) with low-boiling petrol to yield a buff-coloured amorphous powder. This was converted into its fumarate, which was extracted several times with hot ethyl acetate; the extracts were concentrated and cooled. The base obtained from the separated salt was converted into a *methiodide*, which was obtained as a white amorphous powder, sensitive to light, by solution in hot aqueous alcohol (charcoal) and addition of potassium iodide. The yield, based on the original crude picrate, was low. The methiodide had no definite m. p., shrinking from 160° and decomposing at *ca.* 200° (Found : C, 52·0; H, 6·2; N, 6·0; I, 23·1; OMe, 16·2. $C_{22}H_{30}O_4N_2,CH_3I$ requires C, 52·3; H, 6·3; N, 5·3; I, 24·0; 3OMe, 17·6%).

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