346. *Ipecacuanha Alkaloids. Part I. Fractionation Studies and the Isolation of Two New Alkaloids.*

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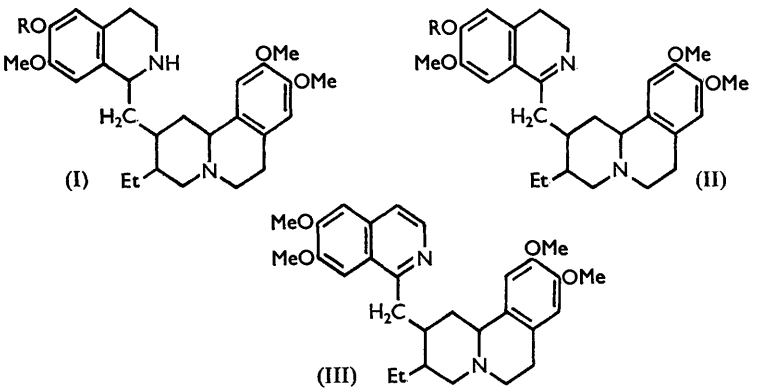
The alkaloids of ipecacuanha have been fractionated by countercurrent distribution. A simple method for the isolation of all previously known non-phenolic alkaloids has been developed and two new alkaloids (protoemetine and ipecac-alkaloid A) have been isolated. The infrared spectrum of *O*-methylpsychotrine has been compared with the spectra of model compounds; it is thereby confirmed that the double bond in this alkaloid is endocyclic.

It has been firmly established that ipecacuanha root contains four other bases in addition to emetine (I; R = Me), the major alkaloid. Paul and Cownley [1] isolated the phenolic alkaloids cephaeline (I; R = H) and psychotrine (II; R = H), to which Pyman [2] added the non-phenolic *O*-methylpsychotrine (II; R = Me) and emetamine (III). All were obtained by fractional crystallisation of the bases or of suitable salts. In addition, Hesse [3] claimed the isolation of two other alkaloids which he named ipecamine and hydroipecamine. Present knowledge of the alkaloids of ipecacuanha supports Pyman's view [2] that Hesse's products were mixtures.



When emetine is isolated from the total non-phenolic alkaloids of ipecacuanha, far more basic material (referred to as " mixed bases " below) remains in the mother-liquor than can be accounted for by the *O*-methylpsychotrine and emetamine present. Large quantities of these mixed bases are obtained as residues from the commercial extraction of emetine, a process which, fortunately for the present work, involves mild conditions throughout. Our interest in the biogenesis of alkaloids prompted us to fractionate these bases by countercurrent distribution with the aim of developing an improved method for the isolation of the known alkaloids and, more importantly, of detecting any new bases. This combination of a large-scale extraction with a powerful fractionation tool made it probable that biogenetic intermediates or substances derived from them, by chance methylation for example, could be isolated.

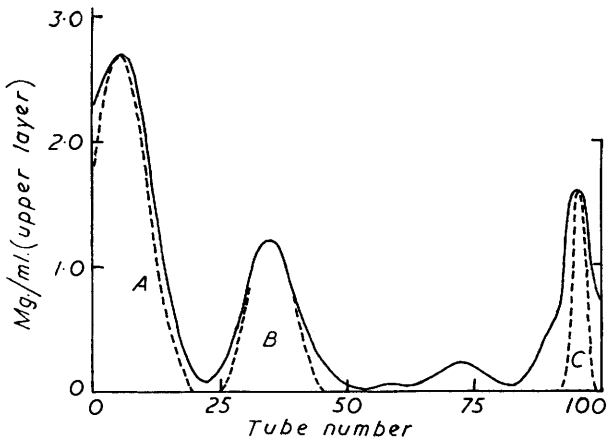
The result of distributing the mixed bases between ethyl acetate and aqueous phosphate buffer (pH 6·4) for 95 transfers is shown in the Figure. Material corresponding to peak *C* yielded emetamine (III) as its crystalline hydrogen oxalate. The width of this peak, compared with the calculated shape, shows clearly that emetamine is not the only weak base in ipecacuanha. *O*-Methylpsychotrine (II; R = Me) was isolated as the hydrogen

[1] Paul and Cownley, *Pharm. J.*, 1894, **25**, 111, 373, 690.
[2] Pyman, *J.*, 1917, **111**, 419; Brindley and Pyman, *J.*, 1927, 1067.
[3] Hesse, *Annalen*, 1914, **405**, 1.

oxalate from material represented by peak *B*, in quantity corresponding to 72% of the total base present, and that represented by peak *A* yielded emetine (I; R = Me) as its crystalline hydrobromide. Thus all the known non-phenolic alkaloids of ipecacuanha can be readily separated in a single distribution. The yield of emetine in different runs only accounted for 20—30% of the base in peak *A* and further investigation of this fraction is planned.

Our main effort was concentrated on the section between peaks *B* and *C* where there were signs of new alkaloids well separated from the known bases. In the large-scale work, a partial separation of the bases was first carried out in a distribution of seven transfers and the desired fraction, contaminated by some *O*-methylpsychotrine, was further fractionated to give a new alkaloid. This was very unstable as the free base and was rapidly destroyed in the presence of aqueous alkali. It did, however, form a stable crystalline perchlorate, $C_{19}H_{27}O_3N,HClO_4$. The yield of pure salt corresponded to 0·002% of the dry ipecacuanha root used for the original extraction. This alkaloid is named protoemetine and its structure is considered in the following paper.

Countercurrent distribution of the ipecacuanha alkaloids. The full curve represents the experimental results, and the broken curve the theoretical values.



A second new alkaloid was isolated from the bases which move rapidly in the distribution experiments. Until sufficient is known about its structure to allow an apt trivial name to be chosen, we designate this base ipecac-alkaloid A. It is crystalline and yields a crystalline oxalate and picrate. The yield as oxalate is only 5×10^{-4}% based upon dry ipecacuanha root so that sufficient quantities have not yet been available to allow its molecular formula to be established with certainty. However, some preliminary work, including analysis of salts, has been carried out. The ultraviolet spectrum of the alkaloid oxalate is characteristic of a veratrole residue and the Zeisel determination establishes the presence of alkoxyl groups. The infrared spectrum of the free base shows no absorption corresponding to >CO, OH or >NH; a band at 1643 cm.$^{-1}$ suggests unsaturation but the alkaloid oxalate was recovered unchanged after attempted mild hydrogenation over platinum. Further progress must await the isolation of greater quantities of this alkaloid.

With pure *O*-methylpsychotrine readily available to us from the fractionation studies, the opportunity has been taken to study further the position of the double bond in this alkaloid. Openshaw and Wood [4] found that the ultraviolet absorption of *O*-methylpsychotrine corresponds to the presence of a 3 : 4-dihydro*iso*quinoline residue. In support of this, the infrared spectrum of the alkaloid shows no absorption in the NH-stretching

[4] Openshaw and Wood, *J.*, 1952, 391.

region whereas emetine absorbs as expected at 3310 cm.$^{-1}$. Also, the former spectrum shows a triplet in the 1550—1650 cm.$^{-1}$ region (see Table). This triplet appears in all the model 3 : 4-dihydro*iso*quinolines examined, including one compound unsubstituted in the 1-position and in which the double bond must necessarily be endocyclic. Only one peak, or in one case a doublet, appears between 1550 and 1650 cm.$^{-1}$ in the spectra of 1 : 2 : 3 : 4-tetrahydro*iso*quinolines. Thus, the sum of the available evidence leaves no doubt that structure (II; R = Me) correctly represents *O*-methylpsychotrine.

Compound	$\nu_{max.}$* (1550—1650 cm.$^{-1}$)
3 : 4-Dihydro-6 : 7-dimethoxy-1-methyl*iso*quinoline	1630, 1607, 1575
1 : 2 : 3 : 4-Tetrahydro-6 : 7-dimethoxy-1-methyl*iso*quinoline	1608
1 : 3-Bis-(3 : 4-dihydro-6 : 7-dimethoxy-1-*iso*quinolyl)propane	1628, 1608, 1578
3-(3 : 4-Dimethoxyphenyl)-3 : 4-dihydro-6 : 7-dimethoxy*iso*quinoline	1622, 1606, 1573
3-(3 : 4-Dimethoxyphenyl)-1 : 2 : 3 : 4-tetrahydro-6 : 7-dimethoxy*iso*quinoline	1610, 1595
1-*cyclo*Hexylmethyl-3 : 4-dihydro-6 : 7-dimethoxy*iso*quinoline[5]	1625, 1612, 1570
6-Benzyloxy-3 : 4-dihydro-7-methoxy*iso*quinoline [5]	1628, 1602, 1576
O-Methylpsychotrine	1618, 1610, 1576
Emetine	1610

* Determined in Nujol mulls with the exception of emetine, which was examined as a film.

Experimental

The small-scale countercurrent distributions (up to 10 g. of base) were carried out in a fully automatic glass machine [6] of 100-tubes (upper and lower phase volumes each 10 ml.) at 21° ± 2°. The tubes were numbered 0, 1, 2 . . . and the upper phase was transferred. Analyses were done by weight,[7] on the upper phase. Solutions in organic solvents were dried (Na_2SO_4) and evaporated at ⩾40° under a reduced pressure of nitrogen.

Analytical samples were dried at 100° over phosphoric oxide *in vacuo* unless otherwise stated.

Small-scale Fractionation of Total Bases.—The mother-liquors from the isolation of emetine from ipecacuanha root (409 kg.) were made alkaline with aqueous ammonia and extracted four times with ethyl acetate. After being washed with water, the organic layers were dried and evaporated, to give a dark brown gum (400 g.). A portion (1·26 g.) was scattered over tubes 0—4 in the machine and then distributed for 95 transfers between ethyl acetate and aqueous buffer made from 0·5M-KH_2PO_4 (5 vol.) and 0·5M-K_2HPO_4 (3 vol.). Analysis showed three major peaks (Figure).

Large-scale Fractionation of Total Bases.—(a) *Isolation of* O-*methylpsychotrine.* The above mixed bases (200 g.) were fractionated in a 7-transfer countercurrent distribution in large bottles between ethyl acetate and the aqueous buffer used in the foregoing experiment. The phase volume was 3 l. and the *lower* layer was transferred. Bottles 2—5 were worked up for bases in the following way. The aqueous layer in each bottle was basified with ammonia and then shaken with the organic layer from the same bottle, followed by two extractions with ethyl acetate (2 × 1 l.). Each organic extract was washed with water (100 ml.), and the combined extracts from the four bottles were dried and evaporated to leave a gum (47 g.). A solution of this gum in ethanol (400 ml.) was treated with hydrated oxalic acid (50 g.) and warmed to give a mother-liquor *A* and a crop of *O*-methylpsychotrine hydrogen oxalate (41 g.), m. p. 162—163° (decomp.), $[\alpha]_D^{18}$ +43·1° (*c* 3·15 in water). Pyman [2] reports m. p. 150—155° (decomp. at 162°), $[\alpha]_D$ +45·9° (*c* 4·26 in water). The *O*-methylpsychotrine base, recovered from the hydrogen oxalate, crystallised from dry ether as prisms, m. p. 121—123° [lit.,[2] m. p. (corr.) 123—124°].

(b) *Isolation of emetamine.* The combined upper layers from bottles 0 and 1 were concentrated to about 2 l. and then shaken with 0·5M-KH_2PO_4 solution (2 × 1 l.). After the organic layer had been washed and dried, it was evaporated to leave the weakly basic alkaloids (13·5 g.) which have been reserved.

The stronger bases (12 g.), recovered as above from the buffer solution, were dissolved together with hydrated oxalic acid (9 g.) in ethanol (100 ml.). Emetamine hydrogen oxalate (0·54 g.) slowly crystallised; it had m. p. 170—171° (decomp.), $[\alpha]_D^{18}$ −5·5° (*c* 13·4 in water). Pyman [2] records m. p. (corr.) 165—171° (decomp.), $[\alpha]_D$ −6·0° (*c* 3·92 in water). Recovery of

[5] Unpublished work from this laboratory.
[6] Craig, Hausmann, Ahrens, and Harfenist, *Analyt. Chem.*, 1951, **23**, 1236.
[7] *Idem, ibid.*, p. 1326.

the base from the hydrogen oxalate followed by crystallisation from ether gave the emetamine–ether complex, m. p. 142—143° (lit.,[2] m. p. 142—143°).

The mother-liquor from the 0·54 g. of hydrogen oxalate above yielded more emetamine when the base therein was distributed between ethyl acetate and aqueous buffer made from 0·5M-KH_2PO_4 (7 vol.) and 0·5M-K_2HPO_4 (1 vol.) for 85 transfers. Three separate runs were made from one-third of the base in each; initially the base was scattered in the first 5 tubes. The emetamine (K 1·9) was recovered as above from tubes 45—70 in each distribution, and the combined base was purified by crystallisation as the hydrogen oxalate (0·47 g.), m. p. and mixed m. p. with the above sample 170—171°.

The alkaloids from tubes 71—90 of the three runs and from the mother-liquor from the isolation of the 0·47 g. crop of emetamine hydrogen oxalate were recovered as Fraction *B* (4·9 g.).

(c) *Isolation of emetine.* Bottles 6 and 7, by the usual working up, gave a dark brown base (65 g.). A portion (13·2 g.) was dissolved in water by the addition of sufficient 2N-hydrochloric acid to make the solution acid to Congo Red. Addition of ammonium bromide caused the very slow separation of emetine hydrobromide tetrahydrate from which the base (3·03 g.) was recovered into ether as usual. A portion of this was benzoylated [8] to give *N*-benzoylemetine, m. p. and mixed m. p. 184—185°.

Emetine base was isolated in better yield from the 65 g. of crude base above by fractionating a portion (1 g.) in a countercurrent distribution between ethyl acetate and aqueous phosphate buffer made from 0·5M-KH_2PO_4 (3 vol.) and 0·5M-K_2HPO_4 (7 vol.). The bases were scattered initially in the first 3 tubes and 95 transfers were applied. Tubes 45—80 contained the emetine, well separated from the other bases; it was isolated as above (0·29 g.). On conversion into the hydrobromide as above, the colourless salt crystallised (395 mg.; m. p. 253—255°, after previous sintering).

Part of the base (28 mg.), recovered from the emetine hydrobromide, was converted into *N*-benzoylemetine (28·4 mg.), m. p. and mixed m. p. 185—186°.

Isolation of Ipecac-alkaloid A.—A portion of the bases (2·7 g.) from fraction *B* [section (b) above] was distributed for 95 transfers between ethyl acetate and aqueous buffer made from 0·5M-KH_2PO_4 (100 vol.) and 0·5M-K_2HPO_4 (6·3 vol.). The bases were scattered initially in the first 5 tubes of the machine. Analysis showed that the bases were spread throughout the machine, rising in amount to a peak at tube 55. Recovery of the bases was therefore carried out arbitrarily from tubes 0—19, 20—40, 41—45, and 46—50. All were treated separately with hydrated oxalic acid in ethanol, and the second and the third fraction yielded crystals (70 mg., 37 mg. respectively). These were combined and recrystallised from ethanol, to give colourless prisms of ipecac-alkaloid A oxalate, m. p. 182—190° (decomp.) after previous sintering (Found: C, 63·7; 63·3; H, 7·3, 6·9; OMe, 21·8%).

Evidence for instability of Ipecac-alkaloid A, or for the presence of a small amount of impurity in the oxalate, was found when the base was converted into its picrate.

The base (28 mg.), recovered from the oxalate (40 mg.) by aqueous ammonia followed by ether-extraction, was treated with picric acid (30 mg.) in ethanol (40 ml.). When the resulting suspension was boiled for a few min., most of the solid remained undissolved. This solid was collected from the cold alcoholic solution, and the filtrate was evaporated to dryness. The dark residual gum crystallised partially from acetone to give rods (5 mg.), m. p. 149—150°. Recrystallisation of the insoluble picrate from aqueous acetone gave ipecac-alkaloid A picrate as rods (22 mg.), m. p. 195—196° (decomp.) [Found: C, 59·1; H, 5·9; N, 8·9%; equiv. (ultraviolet absorption [9]), 794, 780, 754, 781].

Ipecac-alkaloid A base recovered as above from its oxalate (37 mg.) subsequently crystallised from ether as colourless rosettes of needles (22 mg.), m. p. 143—146°, unchanged on further recrystallisation from ether (Found: C, 71·2; H, 8·2; N, 5·6%).

Isolation of Protoemetine.—The mother-liquor A [section (a) above] was evaporated to dryness and the residue dissolved in water (400 ml.). The solution was made alkaline with potassium carbonate, the alkaloids were extracted into ethyl acetate (total 700 ml.), and the solution was thoroughly washed with water. Evaporation of the dried solution left a gum (14 g.). This was divided into halves and these were distributed in two countercurrent distributions of 92 transfers (base scattered initially in the first 8 tubes) between ethyl acetate and

[8] Carr and Pyman, *J.*, 1914, **105**, 1591.
[9] Cunningham, Dawson, and Spring, *J.*, 1951, 2305.

aqueous buffer made from 0·5M-KH_2PO_4 (4 vol.) and 0·5M-K_2HPO_4 (1 vol.). Analysis showed a well-separated peak between tubes 45 and 68. The contents of these tubes from the two runs were combined and the base therein was recovered by the method used above. A brown gum (4·7 g.) was obtained which, as a solution in ethanol (31 ml.) and water (38 ml.), was immediately treated with 60% perchloric acid (6 ml.). The precipitated crystals (4·3 g.) were recrystallised from aqueous ethanol, to give *protoemetine perchlorate monohydrate* (4·1 g.) as pale yellow needles, m. p. 140—142° after previous sintering. Further recrystallisation from aqueous ethanol (charcoal) gave the colourless salt of the same m. p. (loss on drying at 110°, 4·0, 4·6. $C_{19}H_{27}O_3N,HClO_4,H_2O$ requires H_2O, 4·1%. Found on dried salt: C, 54·6, 54·3; H, 6·55, 6·5; N, 3·27, 3·28; Cl, 8·46; OMe, 15·3, 14·2; NMe, 0·94; *C*-methyl, 1·9%; equiv. by titration, 412. $C_{19}H_{27}O_3N,HClO_4$ requires C, 54·6; H, 6·7; N, 3·36; Cl, 8·5; 2OMe, 14·8; 1NMe, 6·95; 1*C*-methyl, 3·6%; equiv., 417·7). The anhydrous salt had m. p. 193—195° and $[\alpha]_D^{25}$ −10·9° (*c* 3·13 in ethanol).

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