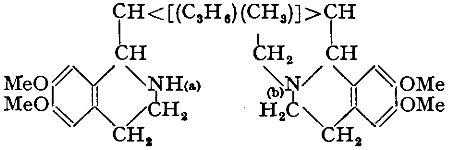
S 12. *Studies on the Structure of Emetine. Part II. Oxidation with Mercuric Acetate, and the Properties of Rubremetinium Salts.*

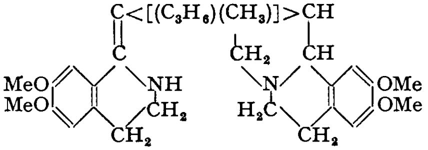
By A. R. Battersby and H. T. Openshaw.

Oxidation of emetine hydrochloride with excess of aqueous mercuric acetate produces rubremetinium chloride in 45% yield. In addition, a small amount of a new, doubly unsaturated base, $C_{29}H_{36}O_4N_2$, has been isolated as the *hydrogen oxalate*. The yield of this base is increased by employing a limited amount of the oxidising agent, and it is shown to be an intermediate in the conversion of emetine into rubremetinium chloride. The quaternary nature of the latter has been confirmed, and its reduction to an unstable dihydro-compound has been observed.

Oxidation of emetine (I) with potassium permanganate leads to extensive breakdown of the molecule, the products isolated being 6 : 7-dimethoxy*iso*quinoline-1-carboxylic acid, corydaldine, and *meta*hemipinic acid (Carr and Pyman, *J.*, 1914, **105**, 1591; Windaus and Hermanns, *Ber.*, 1914, **47**, 1470; Späth and Leithe, *ibid.*, 1927, **60**, 688); with chromic acid, 4 : 5-dimethoxyphthalimide is obtained (Windaus and Hermanns, *loc. cit.*). These and related observations establish the presence of two 1-substituted 6 : 7-dimethoxytetrahydro*iso*quinoline units in emetine, but give no information regarding the structure of the central part of the molecule. The action of mild acidic oxidising agents (aqueous ferric chloride, bromine in chloroform, alcoholic iodine) leaves the carbon skeleton of the alkaloid intact, but effects a dehydrogenation to the red, quaternary rubremetinium salts, $C_{29}H_{33}O_4N_2X$ (X = halogen) (Carr and Pyman, *loc. cit.*; Karrer, *Ber.*, 1916, **49**, 2057; Staub, *Helv. Chim. Acta*, 1927, **10**, 826). *O*-Methylpsychotrine (II) is an intermediate in this reaction, since it has been isolated from the products of interaction of emetine with two atomic proportions of alcoholic iodine, and is further oxidised by bromine to rubremetinium bromide (Pyman, *J.*, 1917, **111**, 419). Dehydrogenation of

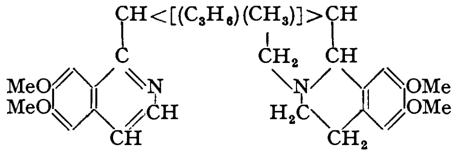


(I.)



(II.)

emetine with palladium, on the other hand, produces the alkaloid emetamine (III) and a fission product, 6 : 7-dimethoxy-1-methyl*iso*quinoline (Ahl and Reichstein, *Helv. Chim. Acta*, 1944, **27**, 366); the different behaviour is probably due to the absence of acidic conditions in this case.



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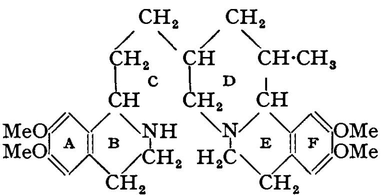

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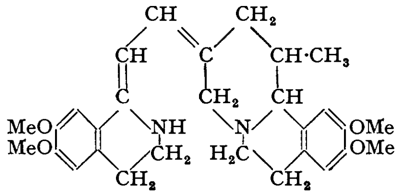

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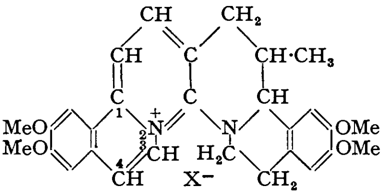
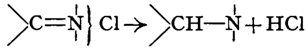


(VI.)

In the hope of obtaining further insight into the mode of oxidation of emetine, we have studied the action of mercuric acetate, a reagent of considerable value for the dehydrogenation of hydroaromatic nitrogenous rings (see, for example, Tafel, *Ber.*, 1892, **25**, 1622; Gadamer, *Arch. Pharm.*, 1915, **253**, 281; 1924, **262**, 452; Legerlotz, *ibid.*, 1918, **256**, 123). The oxidation of emetine with mercuric acetate proceeded readily in boiling, dilute aqueous acetic acid solution, and with excess of the reagent the principal product was rubremetinium chloride, isolated in 45% yield. This is the most convenient method so far recorded for the preparation of rubremetine. A second product of the oxidation was a base, isolated as its crystalline *hydrogen oxalate*. Although this base resembled *O*-methylpsychotrine in the fluorescence of solutions of its salts and the melting point of its hydrogen oxalate, it had a considerably higher specific rotation, and analysis indicated the formula $C_{29}H_{36}O_4N_2$. The presence of two ethylenic linkages was confirmed by micro-hydrogenation, and the absorption spectrum suggests that the double bonds are conjugated with one another and with a benzene ring. The substance may therefore be termed *tetradehydroemetine*, and, on the basis of the emetine formula (IV) of Brindley and Pyman (*J.*, 1927, 1067), it may possess the structure (V). By oxidising emetine with four molecular proportions of mercuric acetate, the yield of tetradehydroemetine was raised to 27%, and very little rubremetinium chloride was produced. The new base was oxidised to rubremetinium chloride by the further action of mercuric acetate, and thus represents a second intermediate stage in the oxidation of emetine. Rubremetinium chloride was found to be relatively stable to hot aqueous mercuric acetate. Exhaustive investigation of the other by-products of the oxidation afforded very small amounts of two crystalline hydriodides, which have not been identified. The remainder of the material was amorphous, and neither unchanged emetine nor *O*-methylpsychotrine could be isolated.

In the formation of rubremetinium chloride from emetine hydrochloride, eight atoms of hydrogen are eliminated, one nitrogen atom becomes quaternary, and the other loses its basic character. Neither *N*-methylemetine nor emetamine (III) gives rise to rubremetinium salts on oxidation (Brindley and Pyman, *loc. cit.*). Any proposed structure for emetine must be capable of accounting for these facts. In arriving at the tentative structure (IV), Brindley and Pyman suggested that the oxidation involved closure of ring C by amidine formation, thus accounting for the mono-acidic, quaternary character of the product, and aromatisation of rings B and C, which would account for the colour of the product. Staub (*loc. cit.*) has already pointed out that the resulting structure (VI) contains two hydrogen atoms fewer than are required by the analytical results. If the C_3–C_4 bond is saturated, it is difficult to account for the intense colour of rubremetinium salts.

In view of the uncertainty concerning the degree of unsaturation of the rubremetinium salts, an investigation of their catalytic hydrogenation has been made, but it has not so far yielded the desired information. Hydrogenation of an alcoholic solution of rubremetinium chloride was very slow, and ceased when about 0·6 mol. of hydrogen had been absorbed. In the presence of sodium acetate, however, reduction was more rapid, and 1·0 mol. of hydrogen was taken up in five hours, the solution becoming almost colourless. The reduction product could not be isolated, as its solution was readily re-oxidised to the orange rubremetinium chloride by atmospheric oxygen, passing through an intermediate green stage, suggestive of a semi-quinonoid system. The beneficial effect of the sodium acetate on the reduction probably indicates that the quaternary nitrogen atom is involved:



In view of the above result, it seemed possible that the pharmacological activity of emetine might involve its oxidation *in vivo* to a rubremetinium salt, which could take part in a biochemical oxidation–reduction system. We are grateful to Mr. L. G. Goodwin, of the Wellcome Research Institution, for undertaking pharmacological tests on a sample of rubremetinium chloride. Activity against *Entamoeba histolytica,* both *in vitro* and *in vivo* (rats), was less than 1/200 that of emetine. Similar results were obtained with *N*-methylemetinetetrahydromethine (Part I, preceding paper).

Statements in the literature concerning the behaviour of rubremetinium salts towards alkali are somewhat contradictory. Karrer (*loc. cit.*) observed that treatment of a solution of rubremetinium iodide with silver oxide gave a halogen-free solution which on evaporation yielded an amorphous, yellow varnish, readily soluble in water to give a neutral solution. Carr and Pyman (*loc. cit.*) found that the halogen-free solution, obtained by the same method, would not yield the base to ether or benzene, but chloroform readily removed it. Evaporation of the chloroform solution gave rubremetinium chloride, the chloride ion evidently being derived from the decomposition of the chloroform by the base. When the aqueous, halogen-free solution was evaporated to small bulk, a yellow gum was precipitated which was insoluble in water but soluble in ether or hydrochloric acid. The aqueous solution remaining after removal of the gum set to a paste of red crystals on cooling.

The opportunity has now been taken to make a reinvestigation of the action of alkalis on rubremetinium chloride, the results obtained being in agreement with those of Carr and Pyman. Potentiometric titration against 0·01N-sodium hydroxide confirms the quaternary nature of the salt. The alkaline solution showed no fall in pH on standing, indicating that no appreciable quantity of pseudo-base is formed under these conditions. When an aqueous solution of rubremetinium chloride was treated with silver oxide, the resulting deep red, halogen-free solution was strongly alkaline, and by acidification of a sample with hydrochloric acid, the chloride was recovered in 88% yield. On evaporation, the solution behaved as described by Carr and Pyman, two products being obtained. One, a bright orange-red microcrystalline solid, was evidently the quaternary hydroxide, since it dissolved in water to give an alkaline solution from which the chloride could be recovered by acidification. This hydroxide is thus much more stable than berberinium hydroxide, which cannot be isolated from its solutions (Perkin, *J.*, 1918, **113**, 503). The second substance was orange-yellow, amorphous, insoluble in water, and soluble in ether. It dissolved in dilute hydrochloric acid, but concentration of the dark red solution yielded only tarry products, and no rubremetinium chloride could be isolated. On basification and extraction with ether, about 20% of an amorphous base was recovered. This second substance is probably a pseudo-base or anhydro-base; its solutions rapidly darken and resinify on standing, and the failure to recover crystalline rubremetinium salts from it is probably due to its instability.

EXPERIMENTAL.

Oxidation of Emetine with Excess of Mercuric Acetate.—Warm solutions of emetine hydrochloride heptahydrate (5 g.) in water (50 c.c.), and of potassium acetate (1 g.), mercuric acetate (21·5 g.), and acetic acid (5 c.c.) in water (100 c.c.), were mixed; a yellow coloration was produced, followed by rapid separation of mercurous acetate. After 1 hour's heating under reflux, the colour of the solution had deepened to that of a concentrated dichromate solution. The mercurous acetate (9·79 g.) was collected and washed successively with water, alcohol, and acetone. The combined filtrate and washings were concentrated to remove alcohol and acetone, treated with a further portion of mercuric acetate (10 g.), and refluxed for 2 hours. On cooling, mercurous acetate (2·34 g.) separated and was collected and washed as above. A finely divided, heavy precipitate of mercurous chloride remained in the flask and was washed by decantation. The filtrate was heated to boiling, and hydrogen sulphide was passed in until the solution had cooled. The precipitated mercuric sulphide was removed by filtration (" Filtercel "), and the filter-cake was dried and extracted with five 200 c.c. portions of boiling alcohol. The extract was concentrated and added to the main filtrate, which was concentrated under reduced pressure. After filtration (charcoal), the solution was treated with concentrated hydrochloric acid; a red, finely divided precipitate then formed. This was redissolved by warming with addition of a small quantity of acetone. On slow cooling, scarlet needles of rubremetinium chloride (1·76 g.) separated, and were collected and dried briefly over sulphuric acid in a vacuum. A second crop (0·20 g.) was obtained from the mother liquor by concentration and further addition of hydrochloric acid. After removal of the second crop of crystals, the mother liquor was shaken twice with ether to remove most of the acetic acid, and then with 7 portions of chloroform; evaporation of the latter extract gave a red resin (0·395 g.) from which a further quantity of rubremetinium chloride (0·105 g.) was obtained by crystallisation from dilute hydrochloric acid; total yield, 2·07 g. (45·6% calculated as hexahydrate). The substance showed similar melting-point behaviour to that described by Carr and Pyman (*loc. cit.*); hydrated material sintered at 103° and became transparent at 128°, whilst material dried at 100° in a vacuum, placed in the bath at 150°, gradually sintered and flowed as the temperature was raised to 190°.

The aqueous layer remaining after the chloroform extraction was boiled to expel dissolved chloroform, cooled, made strongly alkaline with sodium carbonate, and extracted 5 times with ether. When the ethereal extract was dried (Na_2SO_4) a resinous deposit (0·105 g.) was formed, and was removed. Evaporation of the ether left a brown gum (0·888 g.), which was dissolved in hydrobromic acid, concentrated to 10 c.c., and seeded with emetine hydrobromide, but no crystallisation occurred, indicating the almost complete absence of unchanged emetine. When the basic material was recovered from this solution, some black, ether-insoluble tar which formed was removed. The base (0·506 g.) was dissolved in alcohol (1 c.c.) and mixed with a solution of oxalic acid dihydrate (0·3 g.) in alcohol (2·5 c.c.); immediate crystallisation ensued. The material (0·282 g.) on recrystallisation from alcohol (charcoal) formed rosettes of very pale yellow needles (0·194 g.), m. p. 147—149° (decomp.). After 2 further crystallisations from absolute alcohol, *tetradehydroemetine hydrogen oxalate* (82 mg.) was obtained as almost colourless needles, m. p. 151—153° (decomp.), $[\alpha]_D^{15^\circ} + 84{\cdot}5^\circ$ [*c*, 1·9 (calculated as anhydrous substance) in water]. Dilute alcoholic solutions of the substance showed a blue fluorescence. The crystalline material usually became pink or pale brown on drying, this change being accelerated by exposure to light (Found: loss at 100° in a vacuum, 2·3, 2·9. $C_{29}H_{36}O_4N_2,2C_2H_2O_4,H_2O$ requires H_2O, 2·7%. Found, in anhydrous substance: C, 60·2; H, 6·1; N, 4·3. $C_{29}H_{36}O_4N_2,2C_2H_2O_4$ requires C, 60·3; H, 6·1; N, 4·3%). Ultra-violet absorption spectrum in water: Maxima at 2450 A. (ε, 25,600), 3075 A. (ε, 14,900), and 3580 A. (ε, 14,700). Hydrogenation: 21·70 mg. in water (PtO_2) absorbed 1·47 c.c. (1·99 mols.) of hydrogen at 16·9°/746 mm. The reduction product failed to crystallise either as the oxalate or as the hydrobromide.

In another experiment, the aqueous filtrate obtained after removal of the rubremetinium chloride was basified with sodium carbonate and extracted 4 times with chloroform, the extract yielding a dark red-brown tar (0·53 g. from 2 g. of emetine hydrochloride). This partly dissolved in water, and the resulting suspension was extracted continuously with ether. From the extract, a small amount of tetradehydroemetine hydrogen oxalate was obtained. The aqueous phase was concentrated to 5 c.c., and on addition of 1 drop of concentrated hydriodic acid an amorphous mixture of hydriodides was precipitated. By treatment with aqueous acetone a red solid (0·31 g.) was obtained, which was largely amorphous but contained a small amount of a crystalline material in admixture. By washing the solid with warm acetone, and crystallising it from the same solvent (charcoal), rosettes of yellow needles, m. p. 252—254° (decomp.), were obtained, in quantity insufficient for analysis.

Oxidation of Emetine with Four Mols. of Mercuric Acetate.—A solution of emetine hydrochloride heptahydrate (2 g.) in water (20 c.c.) was heated on the steam-bath and treated dropwise with a solution of mercuric acetate (3·8 g.), potassium acetate (0·4 g.), and acetic acid (2 c.c.) in water (40 c.c.) during 1 hour. Heating was continued for a further ½ hour, and, after cooling, the mercurous acetate (1·807 g.) was removed and washed with water until the washings were colourless. After removal of mercury salts from the filtrate with hydrogen sulphide, the clear yellow solution was made strongly alkaline with sodium carbonate and extracted with ether. Evaporation of the extract gave a gum (1·3 g.) which was treated with alcoholic oxalic acid. Crystallisation began after several days, and the mother liquor was then removed by decantation and replaced by a fresh portion of alcohol (10 c.c.). When crystallisation was complete, the tetradehydroemetine hydrogen oxalate (0·533 g.; 27%) was collected, washed with alcohol, and dried in a vacuum at room temperature; m. p. 150—153° with previous sintering. Repeated recrystallisation raised the m. p. to 151—153° (decomp.).

The aqueous solution remaining from the ether extraction was acidified with hydrochloric acid and evaporated to dryness under reduced pressure. The residue was extracted with alcohol and acetone until the extracts were no longer coloured. Evaporation left a red-brown resin (0·258 g.) which was dissolved in water, filtered to remove a little suspended matter, concentrated to 5 c.c., and acidified strongly with hydrochloric acid; a small quantity (6 mg.) of rubremetinium chloride then separated. The mother liquor was submitted to various processes but yielded as the only crystalline material a small additional amount of tetradehydroemetine hydrogen oxalate (14 mg.).

The various alcoholic mother liquors from the purification of the main crop of oxalate were combined, evaporated to dryness, dissolved in water, and made alkaline. The liberated bases were taken up in ether, a quantity of ether-insoluble tar being rejected. The residue from the ethereal extracts (0·310 g.) was dissolved in dilute hydrochloric acid and precipitated by hydriodic acid. The resulting yellowish-brown solid (0·296 g.) was dried in a vacuum and heated under reflux with a quantity of alcohol insufficient to dissolve it completely. After several hours, partial crystallisation occurred and the material was collected; m. p. 227—228° (decomp.) with previous sintering. On crystallisation from water, rosettes of pale yellow needles and rods were obtained, m. p. 222—224° (decomp.) (placed in bath at 150°). Further recrystallisation raised the m. p. to 224·5—226° (decomp.) (Found: C, 54·9; H, 6·1; ash, 0·3%). The quantity of material was insufficient for further purification.

Oxidation of Tetradehydroemetine.—The base (56 mg.), recovered from its hydrogen oxalate, was dissolved in dilute acetic acid (1 c.c.), and mixed with a solution of mercuric acetate (0·14 g.; 4 mols.), potassium acetate (0·025 g.), and acetic acid (0·1 c.c.) in water (2 c.c.). After 3½ hours' heating under reflux there was a considerable deposit of mercurous acetate, and the solution was deep orange. After treatment as described above, scarlet needles of rubremetinium chloride (23 mg.) were obtained which, after being dried in a vacuum over sulphuric acid at room temperature, had m. p. 128—130° (decomp.). In admixture with an authentic specimen (m. p. 121—123°) the m. p. was 124—126° (decomp.). The yield was 32%, calculated as hexahydrate, or 38·5%, calculated as anhydrous salt.

Hydrogenation of Rubremetinium Chloride.—The material employed was dried at room temperature over sulphuric acid in a vacuum, and then contained 2·3% of water. Platinic oxide (20 mg.) was the catalyst in each experiment.

(1) 33·00 Mg. in alcohol absorbed 0·91 c.c. (0·59 mol.) of hydrogen at 15·6°/760 mm. in 24·5 hours; absorption was still proceeding very slowly at the end of this time.

(2) 32·863 Mg. in alcohol containing sodium acetate (30 mg.; 3 mols.) absorbed 1·49 c.c. (1·01 mols.) at 13·5°/766 mm. in 7 hours. After 25 hours, absorption corresponded to 1·12 mols.

(3) 34·361 Mg. as in (2) absorbed 1·59 c.c. (0·99 mol.) at 15·9°/760 mm. in 6 hours.

The solution from the second experiment, which was very pale yellow, began to deepen in colour as soon as air was admitted. It was filtered as rapidly as possible, made alkaline with sodium hydroxide, and evaporated in nitrogen under reduced pressure. A resinous solid remained which was mainly yellow, but was contaminated with a brown substance. It was dissolved in ether, dried (K_2CO_3), and treated with hydrogen chloride. The pale brown solid which was precipitated rapidly darkened and became almost black. No crystalline material could be obtained from it. The solution from the third experiment was left in air, without removal of the catalyst. During 4—5 hours, the colour changed through green to the original orange-red.

Behaviour of Rubremetinium Chloride towards Alkalis.—For the potentiometric titration, a standard "Cambridge" pH meter with glass electrode was used. The curve obtained with rubremetinium chloride was almost identical with that of an equimolar solution of potassium chloride, and differed widely from that of a similar solution of ammonium chloride. The alkaline solution showed no change in pH after 1 hour.

Rubremetinium chloride (0·254 g.) dissolved in water (36 c.c.) to give a solution neutral to litmus. Silver oxide (from 0·085 g. of silver nitrate) was added, and the mixture was shaken, with exclusion of carbon dioxide, until free from chloride ions. The filtered solution, which was a deeper orange than the original, and was alkaline to thymolphthalein, was divided into 3 equal portions. The first portion was acidified with hydrochloric acid and concentrated to a small volume under reduced pressure; rubremetinium chloride (75 mg.; 88%) then crystallised. The second portion was evaporated to dryness in nitrogen under reduced pressure, and the residual red-brown resin (72 mg.) was broken up and extracted with four 15 c.c. portions of boiling ether, the combined extracts being washed twice with a little water. The ether-insoluble material, a bright orange solid, was dissolved in water and added to the aqueous washings. By treatment with hydrochloric acid as before, rubremetinium chloride (25 mg.) was obtained. The yellow ethereal extract was dried (Na_2SO_4); during this process a red deposit formed on the drying agent and on the walls of the flask. The ethereal solution yielded on evaporation a clear orange-yellow gum (39 mg.), which was almost completely soluble in warm dilute hydrochloric acid, giving a deep red solution. On standing, and on concentration, only dark brown gums separated from this solution, which gradually became darker. On basification and extraction with ether, a gum (8 mg.) was recovered; the hydriodide of this failed to crystallise. The third portion of the original solution was concentrated to 2 c.c. by boiling; on cooling, it set to a paste of red crystals. On repeated evaporation with water on the steam-bath, a yellow gum (10 mg.) was formed, similar to that described above. After extraction of this with ether, the aqueous solution was acidified with hydrochloric acid and gave rubremetinium chloride (39 mg.) on concentration.

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