THE PREPARATION OF PORPHYROXINE FROM OPIUM*

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Porphyroxine, a minor alkaloid from opium, is isolated by an extraction procedure, passed through Florisil and alumina (basic and neutral) columns, and eluted with solvents of varying polarity. Porphyroxine from the chloroform and acetone fractions recrystallised from methanol/chloroform in ribbed plates, darkens at 218° and melts at 234–236° (decomp.) corresponding to $C_{22}H_{24}NO_7$, or $C_{23}H_{24}NO_7$. Spectral, and paper chromatographic data of porphyroxine and derivatives are reported.

PORPHYROXINE, a minor alkaloid of opium was first mentioned by Merck (1837). It is distinguished from other alkaloids of the opium poppy (*Papaver somiferum*) by its reactivity with dilute mineral acids which produces a red solution. Dey (1881), Bamford (1930), Fulton (1950, 1952) and Farmilo and Kennett (1950) used this property for the determination of the origin of opium. Hesse (1870), Rakshit (1919) and Machiguchi (1926) claimed to have isolated the alkaloid, but, according to Fulton (1952) had only succeeded in obtaining impure preparations. In Rakshit's claim, it is considered by Rajahgopolan (1945) and Fulton 1952) that codeine, with thebaine, papaverine and a small portion of the red-turning alkaloid were isolated. Fulton (1952) described a method, based mainly on liquid-liquid extraction by which Clair (1956) was able to produce a brown syrupy concentrate of porphyroxine, which did not crystallise. More recently Klayman (1956) reported the isolation of crystalline porphyroxine by liquid-liquid extraction with non-polar solvents and column chromatography. Klayman's method is very time-consuming, for example, a light petroleum (b.p. 30–60°) extraction-step required approximately two months. By combining parts of Fulton's and Klayman's procedures a method has been developed by which a purer porphyroxine may be made in a shorter time.

EXPERIMENTAL

Test for porphyroxine. 2 ml. of extracts were mixed with 5 drops of 2N hydrochloric acid in a small crucible and evaporated to dryness on the boiling water bath; a red colour indicates porphyroxine.

Paper chromatography. All stages of the purification process were checked in isobutanol: acetic acid: water (System 2) (Genest and Farmilo, 1960). The final product was tested in six paper chromatographic systems (Genest and Farmilo, 1961).

Spectra. Ultra-violet spectra of porphyroxine and porphyr (Genest and Farmilo, 1962) were measured with a Beckman DK 2-spectrophotometer and used for assay purposes.

Procedure. The starting material was the residue of an ether extraction prepared by T. & H. Smith, Ltd., Edinburgh, by the K/14-method (Farmilo

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and Kennett, 1953), from 40 lb. of Indian Export opium. This was extracted in light petroleum (b.p. 30–60°), 97 g. of this extracted material was taken up in hot ethanol. The solvent was evaporated until a precipitate was formed. After cooling overnight and filtration, the crystalline precipitate—mostly narcotine—was discarded and the filtrate evaporated to dryness. The residue was taken up in about 140 ml. of dilute acetic acid (3·5 per cent). Sodium acetate (25 g.) were added and the solution extracted continuously with carbon tetrachloride in a liquid-liquid extractor. After about 4 hr. the porphyroxine test was negative. The carbon tetrachloride extract (about 180 ml.) was now shaken out with sodium hydroxide solutions of decreasing concentration; (50 ml. each of 10, 5 and 2 per cent sodium hydroxide) until all porphyroxine was removed. Each alkaline extract was washed with carbon tetrachloride (4 × 50 ml.); and when it became saturated with impurities was refreshed. All sodium hydroxide extracts were pooled, made slightly acidic with acetic acid, then made strongly ammoniacal. The porphyroxine was now exhaustively extracted with chloroform in a liquid-liquid extractor (about 4 hr.). The chloroform extract was evaporated to dryness (1·57 g.). A Florisil (44 g. 60/100 mesh) column (24 × 300 mm.) was packed with the aid of benzene. The resinous chloroform residue was dissolved in benzene: chloroform (4:1), transferred to the column and eluted at 2 ml./min. with the following solvents: 5 × 20 ml. benzene, 1 × 20 ml. benzene:chloroform (1:1), 5 × 20 ml. chloroform, 1 × 20 ml. chloroform:acetone (1:1), 5 × 20 ml. acetone, 1 × 20 ml. acetone:methanol (1:1) and 5 × 20 ml. methanol. Twenty-four fractions of 20 ml. each were collected. Fractions which showed the highest amount of porphyroxine and the least amount of impurities (chloroform and acetone fractions) were combined and the solvent evaporated. An alumina (Woelm, basic) column (12 × 200 mm.) was prepared. Material containing up to 150 mg. porphyroxine can be eluted from this column without too much tailing. For the elution in 10 ml. fractions the same set of solvents as described above was used. Most porphyroxine is eluted in the methanol fractions. The best fractions were rechromatographed several times on alumina (basic or neutral) columns until no more impurities of the now crystalline material could be discovered by paper chromatography. Porphyroxine recrystallised from methanol/chloroform in ribbed plates (73 mg.).

Results

Porporphyroxine has a m.p. 234–236° (decomp.), darkening at 218° (Kofler, 4°/min.).

Found: C, 63·70, 63·90; H, 5·79, 5·60; N, 3·26; CH_3O, 14·60; m.w. 402 (Rast). Calc. for: $C_{22}H_{24}NO_7$: C, 63·76; H, 5·82; N, 3·38; $2CH_3O$, 14·97 per cent; m.w. 414: or $C_{23}H_{24}NO_7$: C, 64·79; H, 5·67; N, 3·29; $2CH_3O$, 14·57 per cent; m.w. 426.

Results of spectral and chromatographic studies were: porphyroxine in ethanol λ_{max} 286·9, 233·9 mμ (ϵ_{max} 8,470, 11,190), λ_{min} 258·1, 227·5 mμ (ϵ_{min} 1,150 and 11,000).

Porphyr hydrochloride, λ_{max} 525, 381, 321·5, 282 mμ (ϵ_{max} 29,040, 7,520, 10,830, and 14,880), λ_{min} 411, 363·5, 302·5, 269·5 mμ (ϵ_{min} 5,450, 7,250, 8,620 and 13,750).

Porphyr phosphate, λ_{max} 535, 378, 323·9, 273·6 mμ (ϵ_{max} 26,850, 8,330, 12,470, and 16,660), λ_{min} at 415, 365, 303, 269·5 mμ (ϵ_{min} 4,890, 6,090, 10,510 and 16,580, respectively). The porphyr compounds were found to be mixtures with $R_{morphine}$ values of 0·60 (violet), 1·17 (red) and 2·76 (colourless). The R_F values of porphyroxine in 6 paper chromatographic systems are 0·69 (isobutanol : acetic acid : water/PO_4) 0·52 (isobutanol :

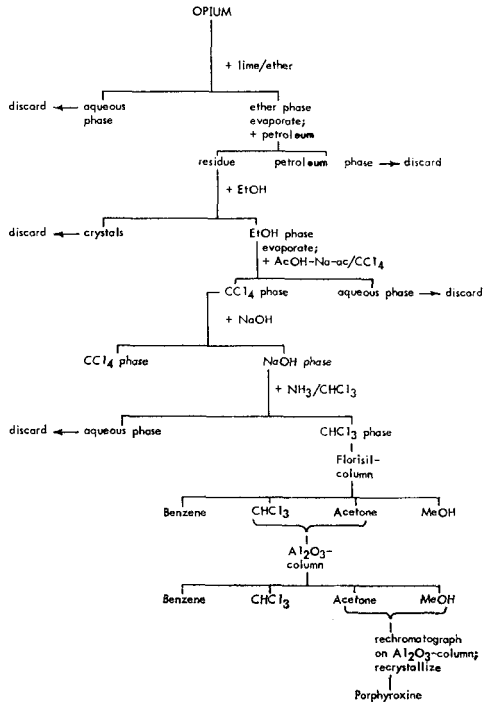

FIG. 1. Flow sheet of procedure for preparation of porphyroxine.

acetic acid : water/SO_4) 0·25 (butylacetate : acetic acid : water/PO_4), 0·83 (propanol : water : diethylamine : paraffin), 0·11 ($HCOONH_4$/s-octanol), 0·02 (paraffin : diethylamine : formamide).

DISCUSSION

The method is outlined in Fig. 1. The starting material contains resins and pigments, a large amount of codeine and thebaine, and much narcotine, papaverine and morphine, some minor alkaloids of phenolic and non-phenolic character, and 0·61 per cent of porphyroxine. In the extraction from buffered acetic acid solution with carbon tetrachloride most of the morphine is left behind in the aqueous phase (Fulton, 1952). The sodium hydroxide extraction, on the other hand, removes only

phenolic alkaloids, leaving behind most of the non-phenolic alkaloids, codeine, thebaine, papaverine and narcotine. According to Fulton (1952) the sodium hydroxide extract contains only porphyroxine, laudanine, a weak base (possibly lanthopine or narcotoline) and a base called alkaloid 7. We found, by paper chromatography, small amounts of all the major alkaloids of opium, in particular codeine. The residue before

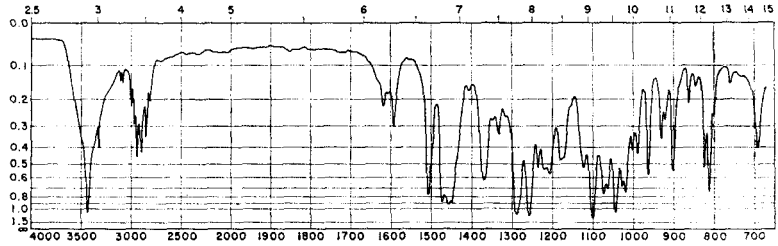

FIG. 2. Infra-red absorption spectrum of porphyroxine (0·5 per cent) in KBr.

application to the Florisil column contained 12·3 per cent porphyroxine, and afterwards, in the best fractions increased to 39 per cent. The final product showed only one spot in five chromatographic systems (Genest and Farmilo, 1961), and gave the R_F values mentioned above. The limit of detection was 0·2 μg. for both the hydrochloric acid (after heating 3 min./100°) and Kiefer's reagents (Farmilo and Genest, 1961), and 20 μg. for the potassium iodoplatinate reagent (Genest and Farmilo, 1960).

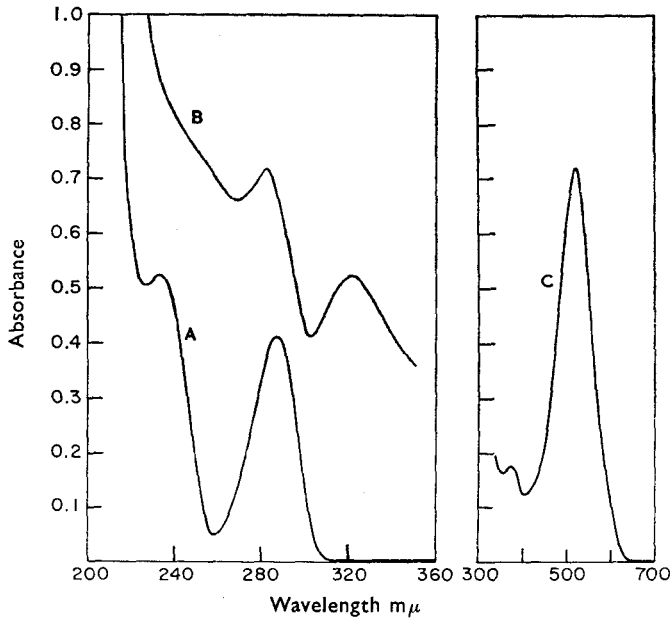

FIG. 3. Ultra-violet absorption spectrum of (A) porphyroxine (0·02 g./litre) in ethanol and of porphyr hydrochloride (B, 0·02; C, 0·01 g./litre) in 67 per cent methanol.

The structure of porphyroxine is still unknown. Klayman (1956) reported the elementary composition to be $C_{21}H_{23}NO_6$ with secondary possibilities of $C_{20}H_{23}NO_6$ and $C_{21}H_{21}NO_6$. Even though the melting point of our preparation is close to that of Klayman's, the elementary analyses of our porphyroxine corresponds to a formula of either $C_{22}H_{24}NO_7$ or $C_{23}H_{24}NO_7$. Analysis shows the presence of two methoxyl groups. Based on its behaviour in alkali, and considering its positive reactions with spray reagents such as *p*-nitroaniline and Kiefer's (Farmilo and Genest, 1961) on the paper chromatogram, porphyroxine has phenolic properties. Spectral results indicate a fair qualitative agreement of absorption maxima in the infra-red, visible and ultra-violet regions (Figs. 2, 3) with those reported by Klayman. The ultra-violet absorptivities reported here are somewhat lower ($\log \epsilon = 3{\cdot}93$ vs. 3·98), while the absorptivities in the visible for the reaction product of porphyroxine with mineral acid, are considerably higher than Klayman's ($\log \epsilon = 4{\cdot}46$ vs. 4·20). The latter's preparation may contain material which absorbs in the ultra-violet but does not produce the characteristic red colour. In a recent paper on the red-turning alkaloids of the papaver family, Pfeifer (1962) also mentioned that Klayman's product probably contained some impurities. Further study of the conditions of the red-colour-reaction is required.

Klayman found that the reaction product of porphyroxine with mineral acid, porphyr hydrochloride, consisted of three components, two coloured and one colourless. He was able to separate these by partition chromatography, but the paper chromatographic separation was incomplete. In the system isobutanol : acetic acid : water (10 : 1 : 2·4) on paper impregnated with sulphate the compounds can be separated from each other by the descending "durchlauf" technique to give the R_{morphine} values cited above. The colourless component gives an elongated spot producing a strong blue fluorescence at 3,660 and 2,537 Å.

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