Rubrine C, a pharmacologically active alkaloid from *Phoradendron rubrum*

SIR,—*Phoradendron rubrum* specie *gracile*, is one of many species of the mistletoe family growing wild in Jamaica. The plant is used in local medicine, mainly as a hypotensive. No mention of this specie has been made in the literature, although there are reports of other species of *Phoradendron* (Turnisped, 1851; Deguy, 1901; Delassus & Gaultier, 1907). Having established the presence of alkaloids in *Phoradendron rubrum*, using the colour reactions with Dragendorff and platinic chloride reagents, we now report the isolation and pharmacological examination of one of these alkaloids.

Preliminary investigations indicated that the compound was either a quaternary ammonium, an *N*-oxide, or amphoteric. Attempts to isolate the alkaloid using standard methods failed, as did techniques using adsorption on alumina. Experiments showed the alkaloid to be highly soluble in polar solvents, whether acid or alkaline, indicating the presence of polar group(s). Whilst these could be carboxylic, hydroxyl or phenolic, chemical tests for phenolic alkaloid were negative. Although compounds containing polar group(s) are not eluted easily from Grade I alumina (Heftman, 1961) because of its strong adsorption, they can be eluted with various solvent mixtures from alumina made less adsorptive by hydration. The following method of isolation was therefore devised.

Whole plant was collected, identified, dried and powdered. The powder was exhaustively extracted with ethanol in a percolator, and the ethanol extract stored overnight in a deep freeze. The proteinaceous materials were precipitated and filtered. The resulting extract, which was a thick syrup, was then dissolved in water before adding basic lead acetate and removing excess lead as sulphide. After adding activated charcoal, the aqueous filtrate was reduced to a syrup under reduced pressure. The syrup was dissolved in methanol and non-alkaloidal crystalline materials were removed and the filtrate reduced to a small volume and chromatographed on a Grade III alumina column. An alkaloid termed "Rubrine C" was eluted from the column with a mixture of methanol and chloroform. The alkaloidal base was a semi-solid and extremely hygroscopic. However, the stable crystalline hydrochloride of this base was obtained after a treatment of a cold mixture of methanol in concentrated hydrochloric acid. The hydrochloride was recrystallized from methanol–acetone mixture and dried at 70°.

Rubrine C hydrochloride had m.p. 250° and was optically inactive. Rubrine C found: C, 38·3; H, 7·9; Cl, 22·9; N, 8·95; O, 22·2%: Calculated for ($C_5H_{13}ClNO_2$) C, 38·8; H, 8·4; Cl, 23·0; N, 9·1; O, 20·7%. Chromatography on paper using the descending technique at 28° with butanol–acetic acid–water (2:3:5) 15 hr gave Rf = 0·4; with butanol–acetic acid–water (4:1:5) 15 hr gave Rf = 0·13; and with chloroform–methanol (1:3) 2·5 hr gave Rf = 0·67. The infrared spectrum of Rubrine C hydrochloride in KBr is shown in Fig. 1. In Nujol there were no peaks additional to those seen with KBr. The nmr spectrum of Rubrine C hydrochloride in D_2O at 25° showed two peaks at τ 6·62 (singlet) and τ 5·69 (singlet) respectively.

Rubrine C caused a fall in blood pressure when doses of 0·5–4 mg/kg were injected intravenously into a cat anaesthetized with chloralose. This fall was not blocked by hexamethonium chloride, or by spinalization, but was partially inhibited by atropine at 2 mg/kg body weight. On the Langendorff rabbit heart preparation, the alkaloid at doses from 10–50 μg decreased the force of the heart beat. This effect was removed by washing and was decreased by atropine. Rubrine C had no effect on the rabbit isolated atria. In the

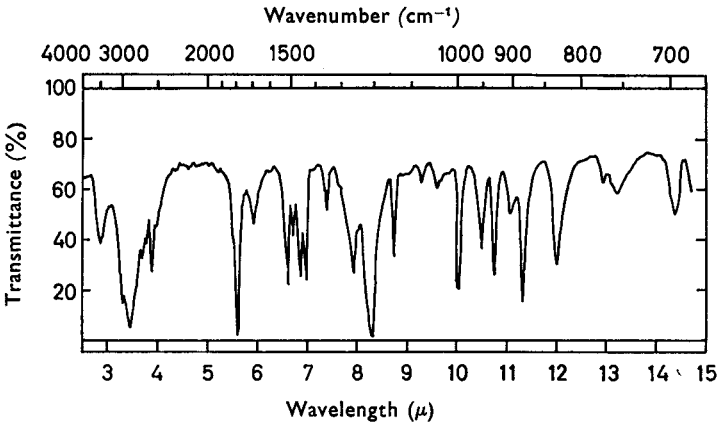

FIG. 1. Infrared spectrum of rubrine C hydrochloride in KBr.

perfused rat hind limb, and rabbit ear, rubrine C caused a prolonged decrease in flow rate at doses of 400 μg. On the guinea-pig ileum, the alkaloid in doses up to 200 μg/ml of bath fluid had no effect. The contractions caused by the spasmogens 5-hydroxytryptamine, nicotine, acetylcholine, barium chloride or histamine were not antagonized or potentiated by that dose of rubrine C.

Rubrine C at 5–40 μg/ml of bath fluid antagonized the contraction of the virgin rat uterus in dioestrus caused by acetylcholine or 5-hydroxytryptamine. Both the pregnant rat uterus and the non-pregnant rat uterus in oestrus, showed sustained contracture of the uterine muscle when the alkaloid, 10 μg/ml of bath fluid, was added. The contracture lasted for about 20 min and prolonged washing was necessary to restore relaxation and spontaneous contractions of the uterus.

This preliminary pharmacology indicates that the activities of this alkaloid are mainly on the cardiovascular system and uterus. From the physical and chemical data this alkaloid might be similar to betaine; however, this is unlikely since betaine is biologically inert, according to Burgen & Hobbiger (1949), and this was confirmed by us on a direct comparison of betaine and rubrine C in various biological tests.

Acknowledgements. We thank Drs. A. W. Sangster and K. L. Stewart from the Department of Chemistry, for preparing and commenting on the infrared and nmr spectra, the Department of Botany for identifying the plant and our technician Mr. C. Smith for his assistance.

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December 19, 1966

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