CONSTITUENTS OF ERYTHREA RAMOSISSIMA (GENTIANACEAE). PART I

S.F. Hussain, M.I. Khattak and S.A. Warsi

North Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Peshawar

(Received March 20, 1968; revised April 25, 1968)

Three glucosides have been isolated as their acetyl derivatives which have been named as acetyl ramosin A, $C_{19}H_{30}O_2$, m.p. 137-80; acetyl ramosin B, $C_{23}H_{30}O_{12}$, m.p. 155-60; and acetyl ramosin C, $C_{23}H_{32}O_{14}$ A hydrocarbon most likely nonacosane and β-sitosterol have also been isolated.

Erythrea ramosissima is found in the plains of north-west parts of West Pakistan at a maximum altitude of 2000 ft. It is a small spring herb with numerous small pink flowers on short pedicels and grows wild in damp soil. Locally, the plant is used to stop nasal bleeding. Dozes of one tablespoon of the whole dried and powdered plant is taken with water when required. The chemical constituents of this plant had not been investigated earlier.

Shade-dried plant was cut in small pieces and percolated several times with ethanol. Solvent was removed completely under vacuum from the combined ethanolic extracts. The dark semi-solid mass, thus obtained, was then extracted with (a) petroleum ether, (b) ethyl acetate, (c) ethanol, and (d) methanol.

Petroleum ether extract (a) was subjected to column chromatography on alumina (M&B). A waxy crystalline substance was obtained in the first few fractions. This, on recrystallisation from acetone yielded a hydrocarbon, m.p. 62–63°. It did not absorb bromine in chloroform, and it formed urea-inclusion compound when boiled with urea in butanol. The adduct dissolved in water on warming, leaving the original hydrocarbon as an oily layer on the surface which solidified on cooling. Absence of unsaturation and formation of urea-inclusion compound indicated it to be a saturated aliphatic hydrocarbon, most likely nonacosane.

Another crystalline compound isolated mainly from later eluate fractions was identified as β-sitosterol by its m.p. 138° and mixed m.p. 137–8°. Further investigation on the eluates of the column was not pursued as none of the fractions yielded any crystalline material.

A portion of the ethanolic extract (c) was acetylated with acetic anhydride and pyridine and chromatographed on alumina. Three glucosides named as (i) acetyl ramosin A, m.p. 137–8°, $C_{19}H_{30}O_{12}$; (ii) acetyl ramosin B, m.p. 155–6°, $C_{23}H_{30}O_{12}$; and (iii) acetyl ramosin C, m.p. 193–4°, $C_{24}H_{32}O_{14}$ were isolated from eluates of the column. All of them yielded glucose on acid hydrolysis which was identified by paper chromatography. The aglycon portion, however, could not be isolated. Estimation of acetyl groups indicated five, four and five acetyl in acetyl ramosin, A, B and C, respectively. IR absorption spectrum of acetyl ramosin A showed absence of unsaturation and a single peak of ester absorption at 1737 cm^{-1} The IR spectra of acetyl ramosin B and acetyl ramosin C have sharp absorptions at 1615, 1700 and 1737 cm^{-1}. The absorptions at 1737 and 1615 cm^{-1} are due to the presence of ester and unsaturation, respectively, in the molecule. The sharp peak at 1700 cm^{-1} is most likely due to the presence of ketone.

NMR spectrum of acetyl ramosin C integrated for 32 protons. This agrees well with the molecular formula ($C_{24}H_{32}O_{14}$) of the compound. Major peaks at 7.95τ and 7.85τ are due to acetyl groups. Peaks at τ 6.2τ and 5.71τ probably indicate the four single protons attached to carbon skeleton of the glucose molecule. A complex pattern is obtained between 5.1 to 4.58τ integrating for ten protons which are most likely the protons of aglycon and the methylene protons of the glucose moiety. A doublet recorded between 2.5τ to 2.32τ possibly indicates the presence of two unsaturated protons of the aglycon.

Experimental

Melting points are uncorrected. Analyses were carried out by Alfred Bernhardt, Max Planck Institut fuer Kohlenforschung Mulheim, Ruhr, Germany. IR spectra were determined on Beckman IR-5 as Nujol mulls. NMR spectrum was determined on Varian DP-60. Petroleum ether was of b. p. 60-80°c.

Dry extract (458 g) obtained by cold percolation of the dried plant (2.4 kg) with ethanol was extracted with petroleum ether (extract A). The residue was then macerated with ethyl acetate which dissolved very little. The large portion (275 g) of the extract was finally extracted with

ethanol (extract B). The final residue was taken up in methanol.

Isolation of Nonacosane.—Petroleum-ether extract A (59 g) was passed through a column of alumina (74×3·5 cm) and eluted with petroleum ether. A light yellow waxy mass was obtained on removal of the solvent from the first fraction. It was dissolved in hot acetone and on cooling gave colourless crystals (2.9 g; 0.12%). It was purified by repeated recrystallisation from acetone, m.p. 62–3°. (Found: C, 85.8; H, 14.1. Calc. for $C_{29}H_{60}$: C, 85.2; H, 14.8%).

Isolation of β-Sitosterol.—Fractions 8 to 12 which were light green to pale white were combined and rechromatographed on alumina using petroleum ether as eluent. Fifteen fractions were collected, out of which only fractions 8 to 13 contained crystalline matter. These were combined and recrystallised from petroleum ether (0.3 g., 0.012%) m.p. 138°, mixed m.p. with β-sitosterol 137-8°C.

Isolation of Glucosides, Acetyl Ramosin A, B and C.—The alcoholic extract B (20 g) was dissolved in acetic anhydride (180 ml) and pyridine (1.0 ml) and refluxed for 2½ hr on an oil bath at 120–30°C. Acetic anhydride and pyridine were completely removed under vacuum by adding small amount of ethanol. The semi-solid mass was extracted with ethyl acetate. The residue left on removal of ethyl acetate was dissolved in benzene and chromatographed on a column of alumina. Forty crystalline fractions were collected which were divided into the following three groups on the basis of their m.ps.

Fractions	*M. p.*	*Group*
1–7	134–35°	A
8–17	145–62°	B
18–40	162–190°	C

Acetyl Ramosin A.—Fractions of group A were combined and recrystallised from benzene (432 mg; 2.16%) m.p. 137–8°, (Found: C, 50.73; H, 6.27; O, 42.95; mol wt, 403 CH_3CO, 48.21, $C_{19}H_{30}O_{12}$ requires: C, 50.66; H, 6.71; O, 42.63; mol wt 450; 5×CH_9CO 47·7%).

Acetyl Ramosin B.—The combined fractions of group B were recrystallised from petroleum ether (0.66 g., 3.3%), m.p. 156–7°, (Found: C, 55.25; H, 5.98; O, 38.38; mol wt, 520; CH_3CO, 33.31 $C_{23}H_{30}O_{12}$ requires: C, 55.41; H, 6.07; O, 38.52; mol wt 498; 4×CH_3CO 34.5%).

Acetyl ramosin C.—The last fractions (melting range of 162–190°) were dissolved in benzene, charcoaled and precipitated by petroleum ether. Crystals thus obtained were further crystallised by ethanol and a little water (0.556 g; 2.77%), m.p. 192–3°. (Found: C, 52.78; H, 5.64; O, 41.84; mol wt, 513; CH_3CO 42.02 $C_{24}H_{32}O_{14}$ requires: C, 52.94; H, 5.92; O 41.14; mol wt 544; 5× CH_3CO, 39·5%.)

Acknowledgement.—The authors wish to thank Dr. G.W. McCrea, Visiting Professor, Department of Chemistry, Peshawar University, for running the IR spectra and Dr. S.H. Zaidi, S.R.O., Central Laboratories, Karachi, for the NMR spectrum.