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CONSTITUENTS OF *CLERODENDRON INFORTUNATUM* (BHAT)—I

ISOLATION OF CLERODOLONE, CLERODONE, CLERODOL AND CLEROSTEROL

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Abstract—Four new crystalline compounds, Clerodolone, Clerodone, Clerodol and a sterol, previously isolated but not characterized and now designated Clerosterol, have been isolated from the root of *Clerodendron Infortunatum* Bhat. Seven sugars namely raffinose, lactose, maltose, sucrose, glactose, glucose and fructose were also identified by paper chromatography of the water soluble portion of the root extract.

Clerodenron infortunatum (Bhat), a shrub, grows freely in the waste lands of East Pakistan. The pinkish-white flowers are foetidly scented and the roots of this plant reported to have vermifuge and anthelmentic properties, are used in chest medication.[1] The leaves are very readily eaten by insects and the name *infortunati* is probably derived from this fate of the plant.

No chemical investigation of the root appears in the chemical literature. The stem and leaves of the plant of Indian origin have been investigated by Bannerjee[2–5] and investigations of other varieties of *Clerodendron* species have been reported by different workers.[6–15] The bitter principle of the leaf material, Clerodin, has been

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investigated by various workers[2-5,16-21] and the structure chemically illucidated by Barton *et al.*[19,20]

Clerodin, $C_{24}H_{30}O_7$ has been reported to have m.p. 164–165°, α_D −47° (*c*, 1·66), E_1^1 18·75 at 202 mμ ν_{max} (Nujol), 1727, 1615, 1252, 738 cm^{-1}; (CCl_4) 3025, 3045 cm^{-1}. Clerodin hemiacetal, m.p. 179–181°, α_D −34° (*c*, 2·35); clerodin hemiacetal anhydride m.p. 210–215°, α_D −67° (*c*, 1·69), ν_{max} (Nujol) 1735, 1250, 1225 cm^{-1};[20] a sterol, m.p. 147–148° and an alcohol, m.p. 75°[4] have also been reported from the leaf and stem materials of this plant.

The present investigation was carried out on an alcoholic extract of the fresh root material. The extract on concentration *in vacuo* precipitated from the aqueous phase a dark green solid which was extracted with petroleum ether and the residue dissolved in hot methanol and purified by repeated crystallization from methanol–water yielding finally colourless needles of clerodolone, m.p. 299–300°.

Clerodolone analyses for $C_{27}H_{44}O_3$ and the UV spectrum, at 202 mμ (ε, 7,800; E_1^1 18·75) shows the presence of an isolated double bond. The compound shows the presence of at least one C—CH_3 and two active hydrogens and on microhydrogenation it absorbs 0·85 mole of hydrogen.

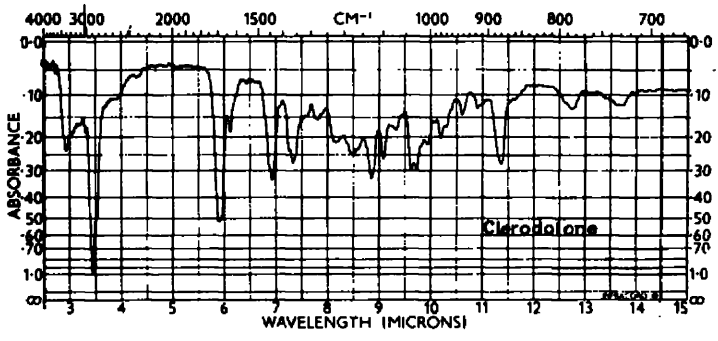

FIG. 1. IR spectrum of clerodolone.

The IR spectrum indicates the presence of alcoholic groups (3400 cm^{-1}), a non-conjugated open chain or six membered ring ketones (1690 cm^{-1}) and a non-conjugated vinyl type double bond (3055, 1645, 885 cm^{-1}). On acetylation it gives a monoacetate, m.p. 270°, with a free hydroxyl group, indicating the tertiary nature of the second hydroxyl group. The semicarbazone has m.p. 315°.

The petroleum ether extract of the residue mentioned above, after chromatography on neutral alumina and elution with benzene gives a very small quantity of needle shaped crystals, m.p. 260° now designated clerodone. It analyses for $C_{29}H_{46}O_2$ and shows a UV maxima at 205 mμ (ε 3,500) and in the IR spectrum shows the presence of a ketone group (1700 cm^{-1}).

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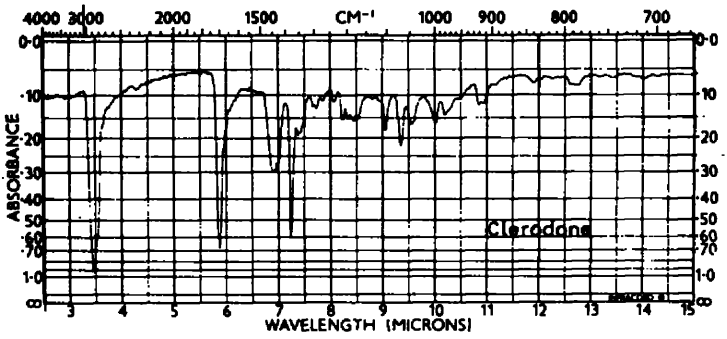

FIG. 2. IR spectrum of clerodone.

The benzene–ether elute (1:1) yields two solids which on crystallization from petroleum ether (60–80°) melt at 205° and 148°.

The higher melting solid, now designated clerodol, m.p. 205°, $\alpha_D^{25} + 36°$ gives only a yellow colour in the Salkowski reaction but a deep pink colour in the Liebermann-Burchard reaction. It analyses for $C_{30}H_{48}O_2$ and shows the presence of an alcoholic group (3400 cm^{-1}) and also unsaturation (1640 cm^{-1}) and has a λ_{max} 203·5 mμ (ε 8,800) in ethanol solution.

The lower melting solid now designated clerosterol, m.p. 148°, $\alpha_D^{24} - 31°$ gives a deep red colour in the Salkowski test and a deep blue colour in the Liebermann-Burchard reaction. It analyses for $C_{28}H_{46}O_2$, has a λ_{max} at 203·6 mμ (ε 9,700) in ethanol solution and shows the presence of an alcoholic group (3400 cm^{-1}) in the IR spectrum.

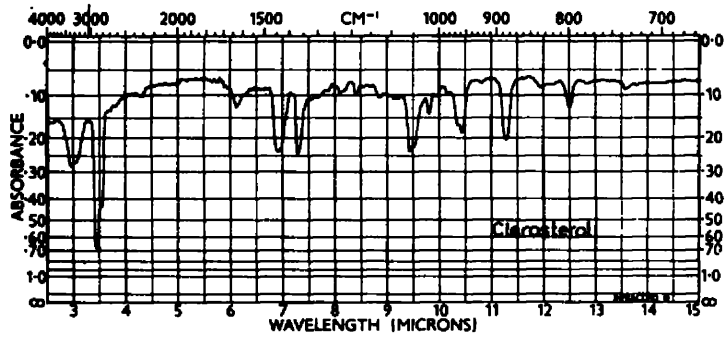

FIG. 3. IR spectrum of clerosterol.

A comparison of the analytical figures and spectroscopic data for clerodolone and clerosterol shows that clerosterol has an additional double bond and possibly has a methyl group in place of the keto group in clerodolone. Otherwise, both compounds are likely to have the same structural configuration and further work on the structure elucidation is now under way.

The aqueous filtrate was subjected to paper chromatography against the known sugars raffinose, maltose, ribose, sucrose, galactose, lactose, glucose, rhamnose, arabinose, fructose, mannose and sorbose. After trial runs with various solvent mixtures

the most suitable solvent was found to be butanol–acetone–water mixture (4:5:1). After identification of the sugars through their *RF* values and specific colour reactions the unknown mixture was chromatographed admixed with the identified components. The number of observed spots remained the same and of the eight spots clearly distinguishable on the paper chromatogram after spraying with silver nitrate reagent, seven could thus be identified as raffinose, lactose, maltose, sucrose, galactose, glucose and fructose.

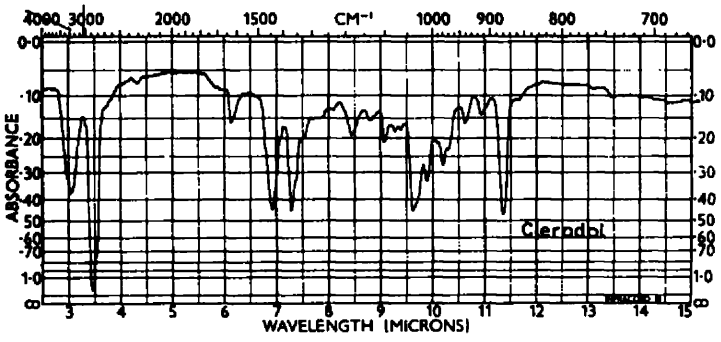

FIG 4. IR spectrum of clerodol.

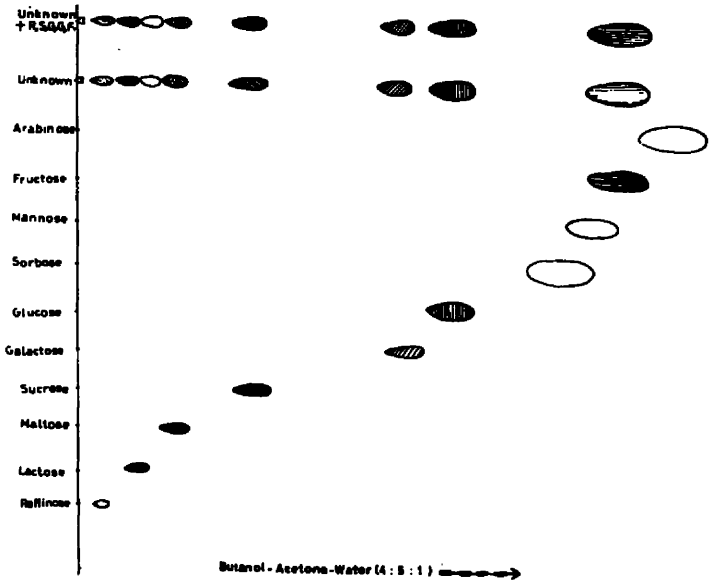

FIG. 5. Paper chromatogram of aqueous filtrate.

Unknown signify aqueous fraction-B; R,S,G,G,F stands for Raffinose, Sucrose, Galactose, Glucose and Fructose. Rhamnose, Ribose and Xylose also included in the mixture flows out of the paper during the running time of 45 hr (as in the photograph above).

EXPERIMENTAL

Analyses were carried out by Messrs. Pascher and Pascher, Microanalytical Laboratories, Bonn, West Germany and Microanalytical Section, Central Laboratories, P.C.S.I.R., Karachi. IR and UV spectra were recorded by Mr. A. Razzaque Qureshi, Drugs and Pharmaceutical Division and Mr. Rahmat Khan, Chemical Research Division, Central Laboratories, P.C.S.I.R., Karachi. All m.ps are uncorrected.

Extraction of fresh roots. Fresh undried roots of *Clerodendron Infortunatum* (12·4 kg; dry weight 9 kg) were cut into small pieces and soaked in rectified spirit (20 l.). After 2 days, the alcohol was collected and the residue re-extracted with alcohol (80%). The combined alcoholic extracts were distilled *in vacuo* and the concentrated solution (ca. 2 l.) allowed to cool in a refrigerator. The dark green gummy precipitate was collected (Fraction A) and the aqueous mother liquor separately investigated (Fraction B).

Isolation of clerodolone. The dark green residue (Fraction A) was repeatedly extracted with pet. ether (60–80°) till the extract was colourless. This solution was then evaporated to give a deep green semi-solid mass (Fraction C). The pet. ether insoluble residue was dissolved in methanol and charcoaled, on repeated crystallization from methanol–water it gave colourless crystals of *clerodolone*, m.p. 299–300°. (Found: C, 77·85, 77·81, 78·07; H, 10·72, 10·47, 10·95; O, 11·71, 11·45; O–CH_3, 0·00; O–AC, 0·00; (C)–CH_3, 3·08; Active H^+, 0·431; Mol. wt. (Rast), 414. $C_{27}H_{44}O_3$ requires: C, 77·83; H, 10·65; O, 11·52; One (C)–CH_3, 3·6; Two active H^+, 0·48%, Mol. wt. 416·6). On microhydrogenation it absorbed 0·85 mole H_2 over Pt. catalyst in glacial acetic acid.

Clerodolone gave a mono-acetate on acetylation overnight at room temp with acetic anhydride in presence of a drop of acetyl chloride. The solvent was removed *in vacuo* and the *mono-acetate* crystallized from dry methanol, m.p. 270°. (Found: C, 76·13, 76·7; H, 9·62, 9·81; O, 14·53; Active H^+, 0·275; (C)–CH_3, 1·7; mol. wt. (Rast) 453, 463. $C_{29}H_{46}O_4$ requires: C, 75·94; H, 10·11; O, 13·9; one active H^+, 0·22; Two (C)-CH_3, 6·67%. Mol. wt. 458·7). It had ν_{max} (KBr) 3500, 3400 (OH), 3050, 2900, 2750 (C–H), 1740 (acetate), 1690 (ketone) and 1645 (unsaturation) cm^{-1}.

Clerodolone gave a crystalline semi-carbazone with sodium acetate and semi-carbazide hydrochloride in ethanol–water, m.p. 315° from ethanol. It had ν_{max} (KBr) 3400, 3300, 3250 i, 3050 i, 2900, 2750, 1700 i, 1680, 1580 cm^{-1}.

Isolation of clerodone, clerosterol and clerodol. The pet. ether extract (Fraction C) was chromatographed on a column of neutral alumina. The initial pet. ether (60–80°) elute gave a dark green solid which did not crystallize. The benzene elute gave a very small quantity of solid which on crystallization from pet. ether (60–80°) gave colourless rhombic needles of *clerodone*, m.p. 260°. (Found: C, 82·04, H, 11·02; O, 7·54; Active H^+, 0·23; mol. wt. (Rast) 380 $C_{29}H_{46}O_2$ requires: C, 81·63; H, 10·87; O, 7·50%; one active H^+, 0·23%; mol. wt. 426·7). On microhydrogenation in glacial acetic acid over Pt. catalyst it absorbed 1·0 mole H_2.

The benzene–ether (1:1) elute first gave a solid which on repeated crystallization from pet. ether (60–80°) gave *Clerodol*. m.p. 205°, α_D^{25} +36° (*c*, 1·0% in ethanol). (Found: C, 82·3, 82·5, 82·25, H, 11·45, 11·15, 11·3; O, 7·4; Active H^+, 0·27; (C)-CH_3, 1·69%. Mol. wt. (Rast) 421. $C_{30}H_{48}O_2$ requires: C, 81·8; H, 11·0; O, 7·3; one active H^+, 0·23; One (C)-CH_3, 3·2%; mol. wt. 440·7). It absorbed 0·9 mole H_2 on microhydrogenation over Pt catalyst in glacial acetic acid. Further elution with the same solvent gave another solid which on crystallization from pet. ether (60–80°) gave *Clerosterol*, m.p. 148°, α_D^{25} − 31° (*c* 1·0% in ethanol). (Found: C, 81·43, 81·8, 81·43; H, 11·44, 10·9, 11·11; O, 7·22; Active H^+, 0·69, 0·403; (C)-CH_3, 10·3; mol. wt. (Rast) 332, 341. $C_{28}H_{46}O_2$ requires: C, 81·10; H, 11·18; O, 7·72; Two active H^+, 0·51; two (C)-CH_3, 8·0%. mol. wt. 390·6). It absorbed 1·75 moles H_2 on microhydrogenation over Pt catalyst in glacial acetic acid solution.

Paper chromatography of aqueous ballast (Fraction B). Different solvents including butanol–acetic acid–water (4:1:5), propanol–ethyl acetate–water (6:1:3) and butanol–acetone–water (4:5:1) were used under varying condition for 16–49 hr at room temp with Whatman No. 1 filter paper. The best solvent for paper chromatography of aqueous fraction B appeared to be butanol–acetone–water (4:5:1), which clearly separated the sugars present in the root material and facilitated their identification. When the spots were developed with silver nitrate reagent and compared with those of known sugars run on the same paper, six of the eight spots so developed could be identified as those of raffinose, lactose, sucrose, galactose, glucose and fructose. In addition a weak spot similar to that of maltose was also noted on the paper chromatogram when run against standard samples of raffinose, lactose, maltose, sorbose, arabinose, fructose, xylose, rhamnose and ribose. The spots for fructose,

sucrose and raffinose was further confirmed by spraying with the specific reagents, naptho-resorcinol and trichloroacetic acid.

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