

Part II

MUHAMMAD NAZIR, IFTIKHAR AHMAD, M. K. BHATTY AND KARIMULLAH

West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore

(Received May 28, 1965)

3,3'-Dimethoxy ellagic acid, succinic acid, and an unidentified compound $C_{19}H_{16}O_7$ containing a number of ester groups have been isolated from *Euphorbia royleana* Boiss.

A systematic study of the chemical constituents of Euphorbeacea is being carried out and the latex of *Euphorbia royleana* Boiss. has already been shown to contain euphol, cycloeucalenol and a novel triterpenoidal alcohol in the previous communication.¹ The present work deals with a detailed study on the juice of the plant which had not been investigated so far.

The scheme given in Fig. 1 was followed in the isolation of various constituents of *Euphorbia royleana* reported in this communication.

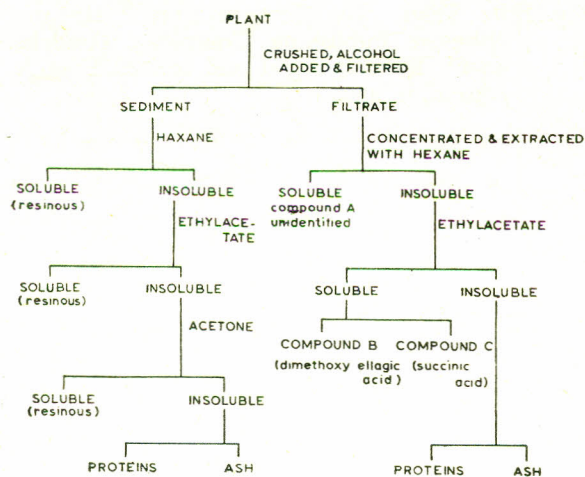


Fig. 1.

Compound C.— $C_4H_6O_4$, m.p. 186-187° is soluble in water and crystallises as needles. It decomposes aqueous sodium bicarbonate. Its infrared spectrum (KBr) shows the presence of carboxylic groups 3560 cm^{-1} , 1750-1700 cm^{-1} and 900 cm^{-1} . The compound on titration against standard alkali proved to be dibasic. The infrared spectrum is superimposable on that of suc-

cinic acid.² Mixed melting point with an authentic sample of succinic acid was undepressed.

Compound B.— $C_{16}H_{10}O_8$, m.p. 335-336 (dec.) pale yellow needles, is insoluble in bicarbonate but is soluble in cold dilute alkalis giving yellow solution from which it can be regenerated on acidification. It absorbs in the infra-red spectrum (KBr) at 1735 cm^{-1} (lactone), at 3450 cm^{-1} (hydroxyl) and at 1108 cm^{-1} (ether). The compound was methylated with diazomethane to a product insoluble in cold dilute alkalis showing the absence of phenolic groups. The infra-red spectrum of the methylated product was identical in all details with that of tetramethoxy ellagic acid.³ The compound was acetylated to give an acetate⁴ as yellow needles m.p. 298-300 — insoluble in cold dilute alkalis. The data, therefore, indicate that compound B is 3,3'-dimethoxy-4,4'-dihydroxy diphenic acid dilactone which can also be called as 3,3'-dimethoxy ellagic acid (also isolated from *Euphorbia formosana* Hay).⁴ The identity of the compound was further confirmed by comparison with a sample synthesised in the Laboratories. Ellagic acid was prepared according to Nierenstein⁵ and acetylated.⁶ The resultant tetraacetate was converted to 3,3'-dimethyl ether of ellagic as described by Jurd.⁷ The IR Spectra of the synthetic and the compound B were identical in all details. Mixed melting point also showed no depression.

Compound A.—Tentative formula $C_{19}H_{16}O_7$, mp. 181-182°, white rhombic crystals, is soluble in chloroform, ethyl acetate, acetone and most of the organic solvents, but insoluble in cold dilute alkalis or mineral acids. In the IR spectrum (CCl_4) it displays bands at 1210 and 1230 cm^{-1} (ester), doublet at 1700 and 1730 cm^{-1} (carbonyl and ester carbonyl) and at 1100 cm^{-1} (ether). The band at 1700 cm^{-1} disappears on reduction with sodium borohydride while bands at 1210 cm^{-1} , 1700 cm^{-1} , 1730 cm^{-1} disappear on reduction with lithium aluminium hydride. The molecular formula of the compound shows sufficient unsaturation but it remains unaffected by bromine in carbon tetrachloride solution. There

* While this work was in progress Sharma et al. isolated taraxerol and ellagic acid from its stem and flowers. (R. C. Sharma, A. Zaman and A.R. Kidwai, Ind. J. Chem., 2, 254 (1964).

was no consumption of monoperphthalic acid even after treatment for 80 hours. In the U.V. it has λ_{\max} (methanol) at $207\text{m}\mu$ ($\log \epsilon=4.43$). This is indicative of a cumulative acetylenic olefin.⁸ Further work on the elucidation of its structure is in progress.

Qualitative analysis of the mineral constituents of the juice and the sediment indicated the presence of sodium, potassium, iron etc. Quantitatively, the inorganic portion of the juice and the sediment contained 11.75% and 7.05% potassium oxide and 17.73% and 6.2% sodium oxide respectively.

Experimental

All melting points are uncorrected and recorded with Fisher-Johns melting point apparatus. The infra-red spectra were recorded with Beckman IR-5A and Perkin Elmer's model 227 spectrometers. Ultraviolet spectra were taken on Beckman D.U. spectrometer. Microanalyses were carried out by A. Bernhardt, Microanalytisches Laboratorium W. Germany. Sodium and potassium as inorganic constituents were determined by a Flame Photometer, Perkin Elmer model 52C.

Isolation of Compound A.—The fresh plant was crushed in roller mills and the juice obtained (16 litres) was preserved with alcohol. The sediment was separated by filtration and the filtrate concentrated below 40° in a cyclone evaporator. The hexane extract of this concentrate after drying over anhydrous sodium sulphate and concentrating yielded colourless rhombic crystals which on repeated crystallisation from the same solvent yielded the compound A (200 mg.) m.p. $181-182^\circ$ (Found: C, 64.68; H, 4.93; Mol. wt. 325. $\text{C}_{19}\text{H}_{16}\text{O}_7$ requires: C, 64.04; H, 4.53; Mol. wt. 356).

Reduction of Compound A.—The compound (82 mg.) in anhydrous ether (40 ml.) lithium aluminium hydride (2 g.) was allowed to react for 2 hours. The excess of lithium aluminium hydride was decomposed with hydrous ether and then with water. The product was extracted eight times with ether (15 ml.). After drying the extract over anhydrous sodium sulphate and evaporating the solvent a white amorphous solid (45 mg.) m.p. $114-119^\circ$ was obtained. λ_{\max} (methanol) $207\text{m}\mu$. IR analysis indicates the elimination

of the carbonyl bands and the formation of the hydroxyl groups.

Acetylation of the Reduced Product.—The reduced compound (35 mg.) in pyridine (5 ml.) and acetic anhydride (2 ml.) was heated on a steam bath for 2 hours. The mixture was thrown into water and then extracted with ether. The extract yielded the acetate (45 mg.) m.p. $201-203^\circ$.

Isolation of Compound B.—The hexane exhausted concentrate was extracted with ethyl acetate. The extract after drying over anhydrous sodium sulphate and concentration deposited a pale brown mass which was taken up in 5% sodium hydroxide. The alkaline solution was acidified with dilute hydrochloric acid and the precipitate extracted with hot ethyl acetate. After removal of the solvent the substance was crystallised from dioxane-methanol to yellow needles of B (200 mg.) m.p. $335-336^\circ$ (dec.) (Found: C, 57.64; H, 3.71; $\text{C}_{16}\text{H}_{10}\text{O}_8$ requires: C, 58.19; H, 3.05).

Acetylation of B.—The compound was acetylated with acetic anhydride—pyridine to yield pale yellow needles m.p. $298-300.4$

Methylation of Compound B.—The compound B (50 mg.) suspended in methanol (20 ml.) was treated with diazomethane solution in ether until a yellow colour persisted. The solvent was evaporated and the pale material crystallised from ethyl acetate to give yellow needles (45 mg.) m.p. $340-342$ (dec.).

Isolation of Compound C.—After removal of the pale brown mass (under isolation of compound B) the mother liquor was evaporated to dryness and the product was found to be soluble in water from which it crystallised in needles (1 g.) m.p. $186-187$ (Found: C, 40.73; H, 4.98; Mol. wt. 121. $\text{C}_4\text{H}_6\text{O}_4$ requires: C, 40.6; H, 5.08; Mol. wt. 118). The compound (10 mg.) in water (25 ml.) was titrated with 0.005 N sodium hydroxide and the basicity was calculated to be 2.

The ethyl acetate exhausted concentrate was evaporated to dryness under "vacuum" over calcium chloride in a desiccator. The dried mass was analysed for its protein content by digesting it with sulphuric acid. The protein content was found to be 1.65%. The dried mass

was further analysed for its ash content which was found to be 24.8%. The ash was analysed, to 11.75% K_2O and 17.73% Na_2O .

The sediment (Fig. 1) was sticky in nature. One hundred gram of it were extracted with hexane in a soxhlet for 15 hours. It gave a greenish yellow sticky mass (31.1 g.) which on charcoal treatment gave a clear yellow solution of the resin.¹

Further extraction of the sediment with ethyl acetate gave (2.6 g.) and acetone (1.3 g.) of the same sticky mass. The exhausted matter was brittle and dark grey in colour. It was insoluble in organic solvents. The protein content of this mass was found to be (8.2%) on the basis of the parent sticky mass and the ash content on the same basis was 12.57%.

The ash was found to contain 7.05% K_2O and 6.2% Na_2O .

Acknowledgement.—The authors wish to express their thanks to the Central Laboratories,

Pakistan Council of Scientific and Industrial Research, Karachi, for supplying the juice.

References

1. M. Nazir, Naeemuddin, I. Ahmad, S.A. Khan, M.K. Bhatti and Karimullah, Pakistan J. Sci. Ind. Res., **8**, 80 (1965).
2. The Sadtler Standard Spectra (Sadtler Research Laboratories 1517 Vine Street, Philadelphia, 2 PA) spectrum No. 11335.
3. *Ibid.*, No. 10064.
4. J. Shinoda and C.P. Kun, J. Pharm. Soc. Japan, **51**, 502 (1931).
5. M. Nierenstein, Helv. Chim. Acta, **14**, 912 (1931).
6. F. Zetsche and M. Graefe, Helv. Chim. Acta, **14**, 241 (1931).
7. L. Jurd, J. Am. Chem. Soc., **81**, 4609 (1959).
8. J.B. Armitage, C.L. Cook, E.R.H. Jones and M.C. Whiting, J. Chem. Soc., 2010 (1952).
9. S.N. Srivastava, D.S. Bhakuni, V.N. Sharma and K.N. Kul, J. Sci. Ind. Res. (India) **21B**, 551 (1962).