CHEMICAL CONSTITUENTS OF EUPHORBIA ROYLEANA BOISS*I

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Euphol, cycloeucalenol and a new triterpenoid alcohol, containing a cyclopropane ring and a vinylidene group, have been isolated from the latex of *Euphorbia royleana Boiss.*

Euphorbia royleana Boiss is locally known as 'Thor'. It is a fleshy thorny shrub or a small plant common on dry and hot rocky slopes of the outer ranges of the Western Himalayas and the Salt Range of West Pakistan. Its milky juice causes dermatitis and is injurious to the eyes.[1] That it possesses cathartic and anthelmintic properties has also been reported.[2] Various [3-13] species of *Euphorbia* have been investigated for their latex constituents. However, since no work appears to have been carried out on this species, its latex has, been systematically examined and the results thereof are reported in this communication.

The scheme given in Fig.1 was followed in the isolation of various constituents of the latex. It follows from the figure that two approaches viz.

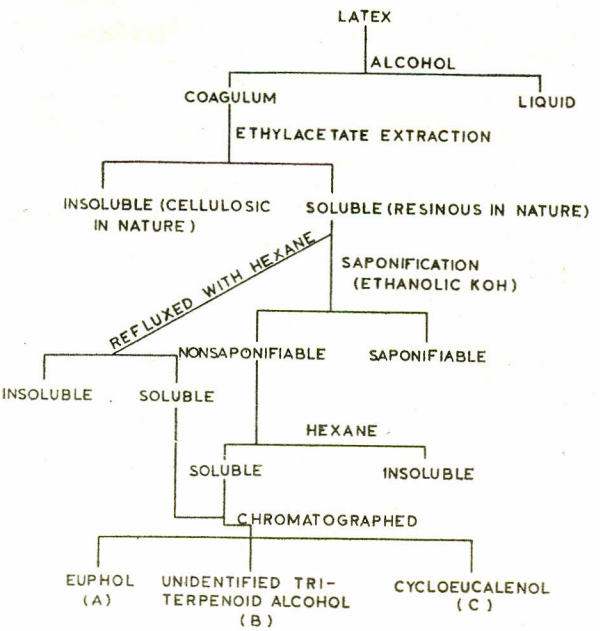

Fig. 1.

(1) saponification and (2) refluxing with hexane of the resinous mass obtained from the latex led to the isolation of the same compounds. Chromatographic analysis on an alumina column of either the non-saponifiable in hexane or the hexane extract of the resin furnished three triterpenes.

Saponification of the resinous mass was carried out with 3 percent ethanolic potash and the non-saponifiable was extracted with ether. A new triterpene alcohol (B) with novel structural features, was isolated for the first time from this nonsaponifiable. In addition, euphol[14] (A) and cycloeucalenol[15] (c) were also isolated from the nonsaponifiable.

In the second approach, the resin as such was refluxed with hexane and the clear extract chromatographed. The fractions obtained were crystallised and further purified by crystallisation of their corresponding benzoates.

Compound A.—$C_{30}H_{50}O$, m.p. 114-115°C. colourless needles, acetate 108-109°C. benzoate 137-138°C. The melting points of the derivatives strongly suggest that the compound is euphol and indeed the infra-red spectrum of the benzoate was identical with that of an authentic sample. Mixed melting point of the two benzoates remained undepressed.

Compound B.—The molecular weight and elemental analysis of the new alcohol correspond to $C_{30}H_{50}O$. The presence of vinylidene group caused doubts whether the compound is C_{30} or C_{31}, as cases of C_{31} triterpenoids having $>C=CH_2$ are known. However, the mass spectrometric analysis indicated the parent molecular ion at 426.

The oxygen function was inferred as that of a hydroxyl group. The secondary nature of this hydroxyl group was established by its oxidation with chromic acid to a ketone which absorbs at 1713-1714 cm^{-1} in the I.R. spectrum (carbonyl in a six-membered ring). Reduction of the ketone with sodium borohydride gave the parent alcohol thereby showing the equatorial nature of the

*While the author's 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).

hydroxyl group. This could also be deduced from the band at 1040 cm^{-1}, in the I.R. spectrum which is characteristic of an equatorial conformation.[16] The alcohol forms a soluble digitonide which means that hydroxyl group is very probably α-oriented. The alcohol acquires red colour, with green fluorescence, in the Liebermann-Burchard test.

With bromine in CCl_4, the compound forms a dibromide. The I.R. spectrum shows bands of medium intensity at 888 cm^{-1} and 1640 cm^{-1} (vinylidene group[17]). These bands disappear on hydrogenation. There appears to be no conjugation of the unsaturation as acetate of the alcohol absorbed at 205 mμ. in the U.V. The N.M.R. spectrum shows a peak at rather high field in the olefinic region[18] at 4.6-4.8 p.p.m. (area=two protons). The deductions from I.R. and N.M.R. were confirmed chemically. The compound on hydroxylation with osmium tetroxide and subsequent lead tetracetate treatment gave formaldehyde.

The presence of a cyclopropane ring was inferred from the shoulder at 3050 [19] cm^{-1} and 1010[20] cm^{-1} in the I.R. spectrum and two equally-spaced doublets at 0.25-0.35 p.p.m. and 0.5-0.62 p.p.m. in the N.M.R. spectrum (J=4 cps).[21] The inference was confirmed chemically by refluxing the hydrogenated product with hydrochloric acid when the two doublets in the N.M.R. disappeared. Further work on the structure of the compound is in progress.

Compound C.—m.p. 138-139°C., colourless needles, acetate m.p. 110°C., benzoate m.p. 130°C., hydrogenated, acetate m.p. 111-112°C. The infra-red spectrum (KBr) displays shoulder at 3050[19] cm^{-1} and band at 1010[20] cm^{-1} (cyclopropane) bands at 890 and 1640 cm^{-1} (vinylidene group[17]). These features are authenticated by N.M.R. spectrum having two doublets at 0.2 and 0.4 p.p.m. together with a peak centred at 4.7 p.p.m. (Olefinic proton region). This evidence indicates that C is cycloeucalenol.

Experimental

All melting points are uncorrected and recorded with Fisher-Johns melting point apparatus. Molecular weights were determined by Rast's method. The infra-red spectra were recorded with Beckman IR-5A and Perkin Elmer's model 227 spectrometers. N.M.R. spectra were run on Varians A-60 spectrometer with tetramethyl silane as the internal reference. Peak positions are downfield in p.p.m. from tetramethylsilane at zero. Microanalysis were carried out by A. Bernhardt Microanalytisches Laboratorium, W. Germany. The latex was supplied by the Central Laboratories, P.C.S.I.R., Karachi.

The latex was coagulated with alcohol and the coagulum separated from the liquid by filtration under suction. The residual solid was exhaustively extracted with boiling ethyl acetate and the extract was dried over anhydrous sodium sulphate. Removal of the solvent yielded yellow sticky mass. This mass (40 g.) was saponified with alcoholic potash (1000 ml.). The alcohol was distilled to half the original volume and then thrown into water. The mixture was extracted with ether to yield a non-saponifiable (36 g.). The non-saponifiable matter (20 g.) was taken up in boiling hexane (1 litre) and the insoluble allowed to settle. The clear supernatant was chromatographed over alumina (BDH 440 g. in a column 45×3.5 cm.). The various fractions were collected and crystallised. Products characterised by acetylation and benzoylation from these fractions are euphol, an unidentified triterpenoid and cycloeucalenol.

In a later experiment, the resin (23 g.) was refluxed with hexane (1200 ml.) for 2 hours and the mixture allowed to settle for a month, and filtered. The filtrate was subjected to alumina chromatography as above and the fractions obtained are shown in Table 1.

TABLE 1.

No.	Solvent	Volume ml.	Weight of substance g.	Product
I	Hexane	3150	0.96	Yellowish gum
II	,,	4500	3·79	White solid
III	,,	1600	2.16	,, ,,
IV	,,	1050	6.40	,, ,,
V	Hexane: Benzene (1:1)	2000	0.59	Colourless needles
VI	,,	2000	0.05	Yellowish gum
VII	Benzene	2000	0.02	Yellowish gum

Attempts to crystallize fraction I, VI and VII failed.

Isolation of Compound A.—Fraction II was crystallised from acetone-methanol to yield needles of euphol, m.p. 114-115°C. (Found: C, 81.26;H, 11.33; mol. wt. 410, $C_{30}H_{50}O$ requires; C, 84.44; H, 11.81; mol. wt. 426) acetate, m.p. 108-109°C; benzoate m.p. and mixed m.p. with an authentic sample 137-138°C.

Isolation of Compound B.—Fractions III and IV on crystallisation either from chloroform-methanol or acetone-methanol gave plates (4 g.) which when

benzoylated yielded needles which on hydrolysis, gave plates of compound B, m.p. 118-119° (Found: C, 84.31; H, 11.54; mol. wt. 426, $C_{30}H_{50}O$ requires C, 84.44; H, 11.81; mol. wt. 426).

Acetylation of Compound B.—Treatment of the compound B (100 mg.) with pyridine (1 ml.) and acetic anhydride (1 ml.) on a steam bath for 2 hours. gave the acetate which crystallised from acetone-methanol as plates (90 mg.) m.p. 108-109°C. (Found: C, 82.42; H, 10.88; mol. wt. 469; $C_{32}H_{52}O_2$ requires C, 82.0; H, 11.2; mol. wt. 468). Benzoylation on similar lines gave benzoate of B, m.p. 161-163°C.

Bromination of Compound B.—The acetate of compound B (0.5 g.) was dissolved in carbon tetrachloride (3 ml.). An excess of bromine solution in carbon tetrachloride (3%) was added dropwise. The product was set aside overnight. After removal of the solvent and unreacted bromine, the mass was crystallised from alcohol to give flakes (510 mg.) m.p. 168-169°C. (Found: C, 61.56; H, 8.04; Br, 25.33; $C_{32}H_{52}O_2Br_2$ requires: C, 61.14; H, 8.37; Br, 25.48).

Hydrogenation of Compound B.—The acetate of the compound B (200 mg.) was dissolved in ethyl acetate (100 ml.) and was added to palladised charcoal in methanol (generated from palladium chloride and sodium borohydride in methanol) and the whole shaken with hydrogen under pressure. After two hours the solution was filtered to remove the catalyst, and the solvents distilled off. The residue was crystallised from acetone-methanol in flakes (190 mg.) m.p. 118-119°C. It was hydrolysed with methanolic potash (3%) or lithium aluminium hydride when it gave the hydrogenated product, m. p. 130-131°C. The same product was obtained directly by hydrogenation of the compound B in a similar manner.

Oxidation of the Hydroxyl Group in Compound B.—The substance (130 mg.) in acetone (15 ml.) was cooled to 0°C. in an ice bath. 8 N chromic acid (0.2 ml.) was added dropwise in one minute. The mixture was stirred for another 2 minutes and the excess of the acid destroyed by the dropwise addition of sulphur dioxide in acetone. The mixture was diluted with water, and extracted with ether. The extract yielded a ketone (100 mg.) m.p. 101-102°C. In the infra-red, it showed the band at 1713-1714 cm^{-1} (Keto in six membered-ring). This ketone (50 mg.) on reduction with sodium borohydride in methanol gave the compound B (40 mg.) m.p. and mixed m.p. 118-119°C.

Hydroxylation of the Double Bond.[22]—The substance B (500 mg.) was taken in ether (20 ml.), and osmium tetroxide (500 mg.) was added. After five days the complex was taken up in methylene chloride, and the solvent evaporated. The residue was refluxed with a mixture of benzene (20 ml.) and water-ethanol (1:1, 20 ml.) containing mannitol (2 g.) and potassium hydroxide (1 g.), for five hours. The mixt ure was diluted with water and extracted with ether. The ether extract yielded white amorphous mass, m.p. 160-165°C. This showed two spots separated slightly in thin layer chromatography on silica gel in benzene ether (50:50). Disappearance of the bands at 1640 cm^{-1} and 888 cm^{-1} in the I.R. spectrum proved the hydroxylation.

Oxidation with Lead Tetraacetate.[22]—The hydroxylated product (450 mg.) in acetic acid (25 ml.) and lead tetraacetate (620 mg.) in acetic acid (25 ml.) was set aside overnight. Water was then added and the product extracted with ether. The water-soluble portion was neutralised with barium carbonate and filtered. The filtrate gave red precipitate with equilibrated ethylene diamine silver chromate solution.[23] The ether extract yielded a ketone in the form of a gummy mass (400 mg.).

Reduction of the Keto to Methylene Group.[24]—The ketone (400 mg.) was dissolved in ethanol (10 ml.) and diethylene glycol (30 ml.). Hydrazine hydrate (3 ml.) was added and the mixture refluxed for two hours. The mixture was cooled and then potassium hydroxide (3 g.) added. The alcohol was distilled off and the reaction mixture heated in an oil bath at 200-210°C., for two hours. The mixture after cooling and dilution with water was extracted with ether. It was crystallised from methanol in flakes, m.p. 85-95°C.

Isomerisation of the Hydrogenated Acetate.[25]—The hydrogenated acetate (200 mg.) was dissolved in acetic acid (5 ml.), and hydrochloric acid (1.5 ml.) added. The solution was heated under reflux for two hours and then poured into water. This was extracted with ether, and the extract washed with sodium bicarbonate. The product, on crystallisation from acetone-methanol, gave needles, m.p. 145-150°C. The mother liquor after drying gave a residue whose m.p. was even lower than 90°C. It showed a number of spots when analysed by thin layer chromatography. A portion of the needles was hydrolysed with lithium aluminium hydride and crystallised from methanol to give needles, m.p. 169-170°C. Reacetylation of the alcohol gave m.p. 148-150°C.

Isolation of Compound C.—Recrystallisation of fraction V from acetone-methanol gave needles, (500 mg.) m.p. 138-139°C. This was acetylated with acetic anhydride pyridine and the product crystallised from acetone-methanol to give needles m.p. 109-110°C. The benzoate m.p. 129-130°C. and the acetate hydrogenated as in the case of compound B to give dihydroacetate m.p. 111-112°C.

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References

1. R.N. Chopra, *Chopra's Indigenous Drugs of India* (U.N. Dhur and Sons Private Ltd., Calcutta-12, 1958), second edition, p. 557.
2. Kirtikar and Basu, *Indian Medicinal Plants* (Lalit Mohan Basu, 49, Leader Road, Allahabad, India, 1933), second edition, Vol. III, p. 2206.
3. N.L. Dutta and Karimullah, J. Sci. Ind. Res., (India), **3,** 212 (1944).
4. Karimullah and R. Gopalchari, J. Sci. Ind. Res., (India), **8B,** 89 (1949).
5. R. Gopalachari and S. Siddiqui, J. Sci. Ind. Res., (India), **8B,** 129 (1949).
6. D.W. Haines and F.L. Warren, J. Chem. Soc., 2554 (1949).
7. A.G. Gonzalez and A. Calero, Anales Real Soc. espan fisy' quim., **45B,** 269 (1949).
8. A.G. Gonzalez and J.L. Breton, Anales Real Soc. espan fisy' quim., **47B,** 363 (1951).
9. A.G. Gonzalez and A.H. Toste, Anales Real Soc. espan. fisy' quim., **48B,** 495 (1952).
10. A.G. Gonzalez and R. Barrera, Publ. Inst. Quim. "Alosona Barba", (Madrid), **10,** 199 (1956).
11. E. Bernatek, A. Nordal and G. Ogner, Medd. Norsk Farm. Selskap, **25** (5) 77 (1963).
12. P. Sengupta and S. Ghosh, Ind. J. Chem., **2,** 298 (1964).
13. V. Anjaneyulu, D.N. Rao and L.R. Row, Current Sci., **33,** 583 (1964).
14. G.T. Newbold and F.S. Spring, J. Chem. Soc., 249 (1944).
15. J.S.C. Cox, F.E. King and F.J. King, J. Chem. Soc., 1384 (1956).
16. J.S.C. Cox, F.E. King and F.J. King, J. Chem. Soc., 1385 (1956).
17. L.J. Bellamy, *The Infra Red Spectra of Complex Molecules* (John Wiley and Sons, Inc., New York), p. 51.
18. K.W. Bentley, *Elucidation of Structure by Physical and Chemical Methods.*—Part I (Inter Science Publishers, New York, 1963), p. 206.
19. K.W. Bentley, *Elucidation of Structure by Physical and Chemical Methods.*—Part I (Inter Science Publishers, New York, 1963), p. 154.
20. A.R.H. Cole, Chem. Ind. (London), 946 (1953).
21. K.W. Bentley, *Elucidation of Structure by Physical and Chemical Methods.*—Part I (Inter Science Publishers, New York, 1963), p. 199.
22. D.W. Haines and F.L. Warren J. Chem. Soc., 2555 (1949).
23. Ph. West and B. Sen, Anal. Chem., **27,** 1460, (1955).
24. D. Arigoni, H. Wyler and O. Jegger, Helv. Chim. Acta, **37,** 1557 (1954).
25. J.S.C. Cox, F.E. King and F.J. King, J. Chem. Soc., 1390 (1956).