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SHORT COMMUNICATION

TRITERPENOID CONSTITUENTS OF *EUPHORBIA CYPARISSIAS*

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Abstract—The non-saponifiable fraction from the light petroleum extract of *Euphorbia cyparissias* has been investigated and shown to contain euphol, 24-methylenecycloartanol, cycloart-23-en-3β,25-diol,β-amyrin, glut-5(6)-en-3-one, glut-5(6)-en-3α-ol, glut-5(6)-en-3β-ol, and β-sitosterol. This is the first recorded instance of glut-5(6)-en-3α-ol occurring in nature.

IN CONNEXION with other studies concerning cypress spurge (*Euphorbia cyparissias*), a noxious weed found in eastern Canada, we have undertaken a reinvestigation of the chemical constituents of this plant. The results of an earlier study[1] were suggestive of the presence of triterpenes known to occur in other *Euphorbia* species. Chemotaxonomic work on the Euphorbiaceae has recently been reviewed[2] and an analytical method presented[2,3] for the rapid identification of the tetracyclic triterpenes in the latex of these plants.

The method employed in the present work was designed to permit the preparative separation and identification of both the tetracyclic and pentacyclic triterpenes present in *E. cyparissias*. However, the unsaponifiable portion of a light petroleum extract of the plant proved to be a mixture of considerable complexity, and success in the resolution was achieved only by repeated chromatography in combination with acetylation procedures. By these means, it was possible to isolate and identify unambiguously the following compounds: euphol, 24-methylenecycloartanol, cycloart-23-en-3β,25-diol,β-amyrin, glut-5(6)-en-3-one, glut-5(6)-en-3α-ol, glut-5(6)-en-3β-ol, and β-sitosterol. These compounds or derivatives thereof were identified by comparison with the relevant authentic specimens. However, neither the 24-methylenecycloartanol nor the derived acetate could be freed from a contaminant which lowered the melting point but other physical characteristics (see Experimental) leave no doubt as to their identity. In addition, two other crystalline substances were obtained but in quantities insufficient to permit complete purification and identification.

Although glut-5(6)-en-3β-ol has been isolated previously[4] from a natural source and more recently[5] from an *Euphorbia* species (*E. royleana*), there appears to be no prior report of the natural occurrence of glut-5(6)-en-3α-ol. This latter alcohol has previously been prepared[6] by lithium aluminium hydride reduction of the corresponding ketone. In the

[1] E. HUPPERT, H. SWIATKOWSKI and J. ZELLNER, *Monatsh.* **48**, 491 (1927).
[2] G. PONSINET and G. OURISSON, *Phytochem.* **4**, 799 (1965).
[3] G. PONSINET and G. OURISSON, *Phytochem.* **4**, 813 (1965).
[4] F. G. FISCHER and N. SEILER, *Ann. chem.* **644**, 162 (1961).
[5] P. SENGUPTA and S. GHOSH, *J. Ind. Chem. Soc.* **42**, 543 (1965).
[6] S. CHAPON and S. DAVID, *Bull. Soc. Chim. France* 333 (1953); J. M. BEATON, F. S. SPRING and R. STEVENSON, *J. Chem. Soc.* 2616 (1955).

present work it was found that sodium borohydride reduction of glut-5(6)-en-3-one followed by acetylation gave, as the main product, glut-5(6)-en-3α-yl acetate together with a small amount of the epimer, glut-5(6)-en-3β-yl acetate, identical with the major pentacyclic triterpenoid consistuent of *E. cyparissias*.

This is the second report of the natural occurrence of cycloart-23-en-3β,25-diol[7] which probably arises biogenetically by oxidation of cycloartenol. Although not detected by us, cycloartenol is a common *Euphorbia* constituent[2] and also would be expected to give rise to 24-methylenecycloartanol.

The wide variety of isoprenoid compounds, which have been isolated, demonstrates the presence, in this plant, of enzymes capable of effecting cyclization of squalene in two different conformations leading to both tetracyclic and pentacyclic systems, and of causing a number of subsequent modifications. The pentacyclic triterpenes present in the *Euphorbia* are undoubtedly as significant taxonomically as the tetracyclic triterpenes being investigated in this regard by the French workers.[2]

EXPERIMENTAL

Melting points were uncorrected and determined on a Köfler hot stage. Alumina was Woelm neutral standardized according to Brockmann. Silica gel (Kieselgel) was used for thin-layer chromatography (TLC). All specific rotations were determined in chloroform. Petrol refers to light petroleum; fractions of b.p. 35–60° used for extraction and chromatography and fraction of b.p. 60–80° used for recrystallization.

Extraction and Isolation of the Non-saponifiable Fraction

Dried ground spurge (410 g) was exhaustively extracted with petrol in a Soxhlet apparatus. On standing the petrol extract deposited a product which showed no triterpenoid characteristics. This substance, m.p. 77–78°, $[\alpha]_D \pm 0°$, is almost certainly the previously reported[1] ceryl alcohol. The clear filtrate was concentrated to afford material (17·1 g) which was refluxed (15 min) in tetrahydrofuran (200 ml) with 10% aq. KOH (25 ml). The non-saponifiable fraction (13·7 g) was isolated from the alkaline solution with ether.

Separation and Identification of Triterpenes

A partial fractionation of the non-saponifiable portion (13·7 g) was achieved by chromatography on alumina (Grade III; 180 g). Successive elution with petrol–benzene mixtures, benzene, and ether afforded the following fractions: (*a*) ketone (1·7 g), (*b*) alcohol (2·3 g), (*c*) alcohol (1·8 g), (*d*) alcohol (0·6 g), (*e*) crystalline alcohol (0·7 g), and (*f*) alcohol (2·3 g). A large fraction consisting apparently of a mixture of hydrocarbons (TLC, i.r.) was removed from the column before the ketone fraction.

Glut-5(6)-en-3-one. Rechromatography of fraction (*a*) on alumina (Grade I; 150 g) and elution with benzene–ether (9:1) yielded a crystalline substance (60 mg), m.p. 237–240°, $[\alpha]_D + 27°$ (*c*, 0·57), ν_{max} 1700 cm^{-1} (in $CHCl_3$) identified as glut-5-en-3-one. Reduction with lithium aluminium hydride gave an alcohol, m.p. 198–201°, which upon acetylation afforded a compound, m.p. 233–236°, identical (TLC, mixed m.p., and i.r.) with authentic glut-5(6)-en-3α-yl acetate.[6]

Fractions (*b*), (*c*), and (*d*) were acetylated since attempts to separate them further proved unsuccessful. Ceryl acetate, formed from ceryl alcohol described above, could be readily

[7] C. DJERASSI and R. MCCRINDLE, *J. Chem. Soc.* 4034 (1962).

separated as it was hydrolyzed by standing for 2 hr on the alumina column (Grade I) whereas the triterpenoid acetates were unchanged.

Careful chromatography of fraction (*b*) on alumina (Grade I; 200 g) with benzene containing increasing amounts of ether afforded, in order of elution, the following triterpenoid acetates, pure after recrystallization: euphyl acetate (283 mg), β-amyrin acetate (trace), glut-5(6)-en-3α-yl acetate (6 mg), and glut-5(6)-en-3β-yl acetate (215 mg). Similarly, fraction (*c*) afforded the following acetates: β-amyrin acetate (32 mg), 24-methylenecycloartanyl acetate (552 mg), and glut-5(6)-en-3α-yl acetate (10 mg). Fraction (*d*) was chromatographed over alumina (Grade III; 64 g) and elution with petrol gave fractions which were recrystallized to afford the following: β-amyrin acetate (27 mg), 24-methylenecycloartanyl acetate (195 mg), and an unknown acetate (3 mg; m.p. 150–154°). The identity of these acetates was demonstrated as described below.

Euphyl acetate. Recrystallization from chloroform–acetonitrile yielded needles, m.p. 106–107°, $[\alpha]_D$ +40° (*c*, 1·17), which upon hydrolysis gave material, m.p. 112–114° (ether–methanol), $[\alpha]_D$ +34° (*c*, 1·15). Treatment with benzoyl chloride–pyridine gave a benzoate recrystallized from ether–alcohol to afford euphyl benzoate, m.p. 135–137°, identified by direct comparison (TLC, i.r., mixed m.p.) with an authentic specimen.

β-Amyrin acetate. Recrystallization from ether–petrol gave material, m.p. 239–241°, undepressed upon admixture with authentic β-amyrin acetate. Comparison of i.r. spectra further confirmed the identity.

Glut-5(6)-en-3α-yl acetate. Recrystallization from chloroform–methanol gave needles, m.p. 236–237°, identical (TLC, i.r., mixed m.p.) with authentic glut-5(6)-en-3α-yl acetate.

Glut-5(6)-en-3β-yl acetate. Recrystallization from ether–petrol afforded crystals, m.p. 190–191°, undepressed upon admixture with glut-5(6)-en-3β-yl acetate prepared as described below.

Glut-5(6)-en-3-one (60 mg) in tetrahydrofuran (6 ml) and alcohol (2 ml) was treated with excess sodium borohydride for 20 min at room temperature. The product, shown by thin-layer chromatography to be a mixture of alcohols, was acetylated (acetic anhydride–pyridine) and chromatographed carefully on alumina (Grade I; 10 g). Benzene–ether (19:1) eluted a substance (56 mg) shown to be glut-5(6)-en-3α-yl acetate described above. Further elution with the same solvent gave a chromatographically (TLC) pure fraction (3 mg) recrystallized from ether–petrol to afford glut-5(6)-en-3β-yl acetate, m.p. 184–187°.

24-Methylenecycloartanyl acetate. Recrystallization from ether–acetone gave material, $[\alpha]_D$ +56° (*c*, 1·37), ν_{max} 1640, 890 cm^{-1} (in $CHCl_3$), softening in the range 80–100° and melting after resetting at 107–109°. On some occasions crystalline material, m.p. 111–113°, could be obtained by recrystallization from chloroform–alcohol. The NMR spectrum,* showing bands at τ 9·14, 9·08, 9·03 for the methyl groups and τ 9·71, 9·64, 9·46, 9·37 multiplet attributed to a cyclopropane grouping, was very similar to that reported[2] for 24-methylenecycloartanyl acetate. No melting point depression was observed upon admixture with authentic 24-methylenecycloartanyl acetate

Hydrolysis gave material, m.p. 109–113°, $[\alpha]_D$ +44° (*c*, 0·67) (Found: C, 84·55; H, 11·53. Calc. for $C_{31}H_{52}O$: C, 84·48; H, 11·89%). Admixture with authentic 24-methylenecycloartanol (m.p. 121–122°) showed no melting point depression although the melting point of this substance was lower. Sublimation *in vacuo* did not raise the melting point.

β-Sitosterol. Fraction (*e*) on recrystallization from chloroform–methanol gave β-sitosterol, m.p. and mixed m.p. 137–138°. Benzoylation yielded β-sitosterol benzoate, m.p.

* Determined in $CDCl_3$ solution using a Varian A-60 spectrometer with TMS as an internal standard.

and mixed m.p. 144–146° (acetone–methanol). Further proof of the identity was obtained from comparison of i.r. spectra and TLC behavior.

Cycloart-23-ene-3β,25-diol. Fraction (*f*) was chromatographed on alumina (Grade III; 117 g) and elution with benzene–ether (9:1) gave a fraction recrystallized from chloroform–petrol to afford an alcohol (40 mg), m.p. 198–201°, $[\alpha]_D$+40° (*c*, 0·56) (monoacetate, m.p. 147–149°), exhibiting no melting point depression upon admixture with cycloart-23-ene-3β,25-diol.[7] Eluted slightly earlier than this compound was another substance, recrystallized from ether–alcohol as plates, m.p. 149–163°, of insufficient quantity to permit further purification and characterization.

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