Steroid, triterpenoid, and flavonoid constituents of Euphorbia pulcherrima Willd. leaves

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Light petroleum, ether, and alcoholic extracts of Euphorbia pulcherrima Willd. leaves, yielded 3 triterpenes, 2 steroids and 3 flavonoids. By using UV, IR, NMR, and MS spectral methods as well as available standard sample comparison, the compounds were found to be germanicol acetate (yield 0.25%), germanicol (0.025%), brein (0.0054%), β-sitosterol (0.042%), β-sitosteryl 3-D-glucoside (0.01%), kaempferol 3-O-glucoside (0.013%), kaempferol 3-O-rutinoside (0.11%) and rutin (0.012%). The first two compounds exhibited moultng activity when assayed on Spodoptera littoralis Bosid. (cotton leaf worm).

Euphorbia pulcherrima Willd., »Christmas flower«, is an ornamental plant widely cultivated in Egypt (1, 2, 3). It was reported that all parts of the plant are poisonous and that the bracts have insecticidal properties (1, 4, 5). The literature encompasses scattered chemical studies, carried out abroad, on the latex, stems, bracts and flowers (2, 6, 7, 8). However, no publications could be found dealing with the chemistry of the leaves, although it has been reported (4) that a small child died after eating a single leaf.

This study of the leaves was undertaken as a result of a preliminary examination of the light petroleum extract which showed to be just as potent as the insect-moulting hormones, when assayed on Spodoptera littoralis Bosid. (cotton leaf worm).

EXPERIMENTAL

Plant material

The plant was collected from cultivated areas in several public and special gardens in Alexandria. It was identified as Euphorbia pulcherrima Willd. by the late professor Dr. V. Täckholm.

Isolation procedure

2.5 kg of the powdered leaves were successively extracted, at room temperature, with light petroleum (50—70 °C), ether, and ethanol (95%).
Light petroleum extract

Refrigeration of the light petroleum extract (110 g) furnished a yellowish-white deposit that was repeatedly crystallised from methanol to give compound »A« m.p. 279—80 °C, (yield 0.25%). The light petroleum mother liquor was saponified with 20% alcoholic potash. Part of the unsaponifiable matter (17 g) was chromatographed on an alumina column (Merck, 400 g) and eluted, in succession, with benzene (fractions 1—58, 200 ml each), then with 1% methanol in benzene (fractions 59—71). Separate crystallisation of material from fractions (27—58), (60—65) and (69—71), respectively, from methanol, gave the following products: compound »B«, m.p. 172—4 °C (yield 0.275%), β-sitosterol (yield 0.042%), and compound »D«, m.p. 222—4 °C (yield 0.0054%).

Ethereal extract

The ethereal extract gave, on concentration, a greenish deposit which was repeatedly crystallised from ethanol to give β-sitosteryl 3-D-glucoside, m.p. 284—6 °C (yield 0.01%).

Alcoholic extract

The extract was freed from inorganic material and the solvent was evaporated to give 294 g of a dark brown residue; 35 g of this residue were chromatographed on a silica gel column (Chemapol, 700 g). The column was eluted with EtOAc (fractions 1—29, 200 ml each) then with 5% methanol in EtOAc (fractions 30—75). Separate crystallisation of material from fractions (30—45) and (63—75), respectively, from methanol gave 2 flavonoids identified as kaempferol 3-O-glucoside, m.p. 229—31 °C (yield 0.013%) and rutin, m.p. 183—5 °C (yield 0.012%) whereas crystallisation of fractions (56—61) from distilled water gave a flavonoid »G«, m.p. 176—8 °C (yield 0.11%).

Characterisation and identification of the isolated compounds:

β-Sitosterol, β-sitosteryl 3-D-glucoside, kaempferol 3-O-glucoside and rutin were identified by direct comparison of mp's, mmp's, with reference samples TL chromatograms, IR and UV spectra.

**Compound »A«:** m.p. 278—80 °C (MeOH), yield 0.25%. Gives a bright red colour in Liebermann's and Salkowski's tests. IR (KCl cm⁻¹): 1718 (C = O of an ester), 1640 (C = C), 1450, 1385, 1375 (gem-dimethyl), 1242 (C-O-C of acetate), 840 and 820 (cyclic compound). Ms, m/e: M⁺ 468, 453 (M⁺ — CH₃), 408 (M⁺ — acetic), 393, 218, 207, 205, 204, 203, 190, 189, 177. NMR (δ ppm 60 MHz, CCl₄): singlets at 0.7, 0.78, 0.88, 0.95 and 1.04 (24 H, 8 × CH₃), 1.4 ~ 1.6 (20 H, 10 × CH₂), 1.94 (3H, OCOCH₃), multiplet at 4.05 (1 proton next to acetyl group), multiplet at 4.82 (H, vinylic proton).

**Compound »B«:** m.p. 172—4 °C (MeOH), yield 0.025%. Gives a bright red colour in Liebermann's and Salkowski's tests. IR (KCl cm⁻¹): 3350 (OH), 1640 (C = C), 1450, 1380, 1370 (gem-dimethyl). Ms, m/e: M⁺ 426, 411 (M⁺ — CH₃), 408 (M⁺ — H₂O), 218, 207, 205, 204, 203, 190, 189, 177. NMR (δ ppm, 60 MH₂, CCl₄): 0.64, 0.7, 0.85, 0.9 and 1.02 (24 H, 8 × CH₃), 1.18, 1.7 (20 H, 10 × CH₂) and a multiplet at 4.8 (H, vinylic proton). Compound »B« was detected in the light petroleum extract before saponification, but it was isolated afterwards from the unsaponifiable matter. Acetylation of compound »B«: gave crystals m.p. 279—80 °C, that were found to be identical with com-
pound »A«. The signals in the NMR spectra of compounds »A« and »B« between 0.64 and 1.04, attributed to 8 overlapping methyl groups, as well as the band characteristic of gem-dimethyl groups in the IR, were good evidence for a pentacyclic triterpenoid constitution. The molecular ion appearing in the Ms of compound »B« at m/e 426, indicated a monohydroxy triterpenoid skeleton (C_{30}H_{50}O). The Ms showed the diagnostically important peaks that characterise the oleanene series and particularly the Δ-18 group (9). Moreover, the multiplet in the NMR at δ 4.8 and the band in the IR at 1640 cm\(^{-1}\) confirmed the presence of a nuclear double bond. In the Ms, cleavage of ring C yielded the ion m/e 207 (23%), enclosing rings D and E, characteristic for 3-hydroxy pentacyclic triterpenes. This ion was accompanied by the ion m/e 218 (85%) comprising rings D and E. A conclusive proof of the structure was obtained from the highly pronounced signal at m/e 177 (73%) characteristic for Δ-18 oleanenes.

By comparing the above findings with those published (10) for Δ-18 oleanene compounds, material »B« was found to be identical with germanicol*.

The Ms of compound »A« showed a molecular ion at m/e 468, followed by peaks at m/e 453 (25%), 408 (4%) and 393 (8%) due to loss of methyl, acetyl and combination of both. Saponification of material »A« yielded crystals, m.p. 172—4 °C, identical with compound »B«. Accordingly compound »A« is germanicol acetate.

**Compound »D«:** m.p. 220—1 °C (MeOH), yield 0.0054%. Gives positive Liebermann’s and Salkowski’s tests; acetyl deriv., m.p. 198—9 °C (MeOH).

IR (KBr cm\(^{-1}\)): 3340—3420 (OH), 1640—1660 (C = C), 1470, 1410, 1385, 1360 (gem-dimethyl), 1040 (-C-O), 1000, 975, 870, 850 and 830. Ms, m/e: M\(^+\) 442, 427 (M\(^+\) — CH\(_3\)), 424 (M\(^+\) — H\(_2\)O), 409 (M\(^+\) — CH\(_3\) + H\(_2\)O), 406 (M\(^+\) — 2H\(_2\)O), 391, 301, 273, 271, 257, 255, 234 (100%), 219, 218, 216, 207, 205, 191, 189.

The colour tests, as well as the bands in the IR at 1385, 1360 and 1660—1640 cm\(^{-1}\), indicated an unsaturated triterpenoid structure. The Ms showed a mol. ion at m/e 442, pointing to a dihydroxy triterpene, the spectrum showed in addition the diagnostically important peaks, that characterise Δ-12 pentacyclic triterpenes, resulting from a retro-Diels-Alder fragmentation (9). The appearance of the highly pronounced peak m/e 191 (84%) in comparison with that at m/e 203 (12%) indicated that compound »D« is closely related to the ursene series (11). The strong band in the IR at 3340—3420 cm\(^{-1}\) as well as the ease of acetylation and formation of a crystalline derivative confirm that the oxygen atoms make part of two hydroxy groups, which are either primary and/or equatorially oriented secondary alcohols. By analogy to all other triterpenes of the α-amyrin series, one of the oxygens might be attached at C-3. The position of the other is suggested by the Ms spectrum. The high intensity base peak at m/e 234 (100%) giving rise to peaks at m/e 219, 218 and 203, as a result of sequential loss of hydroxy and methyl groups, shows that this hydroxy group is present either in the form of a hydroxymethylene group at C-29 or 30 in ring E, or at C-17 in ring D, or as separate hydroxy and methyl groups. The absence of a mass peak at M\(^+\) — 31 characteristic for a loss of -CH\(_2\)OH excludes the possibility of the occurrence of the

* An authentic sample of germanicol was not available for direct comparison.
-CH₃ and -OH as -CH₂OH. Accordingly, OH and CH₃ should occupy different locations. The low intensity ion peak m/e 203 (12%) confirms that the C-17 substituent is a methyl group. Therefore, the second hydroxyl might be at C-16, 21 or 22. Referring to literature, one may suggest that compound »D« might be similar to brein (12) (3,21-dihydroxy ursan-12-ene), m.p. 216–7 °C, acetyl deriv. m.p. 196 °C.

**Compound »G«**: m.p. 176–8 °C (H₂O), yield 0.11%. This compound is a flavonoid glycoside; gave a green colour with FeCl₃ T.S. and magenta red with Mg/HCl. Under UV light it appears as a purple spot changing to yellow when treated with ammonia vapours. UV max. (in MeOH): 270, 300, 370 nm; UV shifts after addition of NaOME: 280, 330, 405; AlCl₃: 280, 305, 350, 400; AlCl₃/HCl: 280, 305, 350, 400; Na OAC: 280, 310, 390; Na OAC/H₃ BO₃: 270, 305, 357. Acid hydrolysis yielded glucose, rhamnose and an aglycone identified as kaempferol by direct comparison with a reference sample. The UV of both aglycone and glycoside indicated that the sugar is linked at C-3. NMR (6 ppm, DMSO-d₆): 1 (S, 3H, CH₃); 6.6, 6.4 (2 d, 2H, J = 2.51, H-8, 14-6); 8.2, 7.03 (2 d, 2H, J = 8.5 Hz, H-2,6, H-3.5). The 2 signals attributed to C-1 sugar protons (5.8 ~ 5.5 and 4.5 ppm) suggested that it is a diglycoside which was confirmed by acid hydrolysis. Moreover, that appearance of a strong signal at 8 1.0 for 3 protons confirmed that the sugar part is rhamnose. It was concluded that the sugar is a 3-O rutinoside from the chemical shift in the NMR observed for C-1 and C-CH₃ signals. Therefore, compound »G« is kaempferol 3-O rutinoside.

**Moulting activity of germanicol and its acetate (compounds »A« & »B«):**

Topical application** of germanicol and its acetate (in hexane) on the dorsum of the thorax of the 6th instar larvae of Spodoptera littoralis Bosid. (cotton-leaf worm) showed that 22 and 51% respectively, pupae failed to develop into adult form. The effective doses were found to be 3 µg of germanicol and 1 µg of germanicol acetate.

**REFERENCES**


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IZVOD

Steroidi, triterpenoidi i flavonoidi u lišću Euphorbia pulcherrima Willd.

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Eterski, petroleterski i alkoholni ekstrakti lišća Euphorbia pulcherrima Willd. sadrže 3 terpena, 2 steroida i 3 flavonoida. Primjenom spektroskopskih metoda (UV, IR, NMR i MS) utvrđene su strukture sljedećih spojeva: germanikol-acetat (sadržaj 0.25%), germanikol (0.025%), brein (0.0054%) β-sitosterol (0.042%), β-sitosteril-3-D-glukozid (0.01%), kemferol-3-O-glukozid (0.013%), kemferol-3-O-rutinozid (0.11%) i rutin (0.012%). Ispitivanja germanikola i germanikol acetata pokazala su da obje supstancije djeluju na mitarenje Spodoptera littoralis.

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