Chemical Study of the Latex, Stems, Bracts, and Flowers of "Christmas Flower" (*Euphorbia pulcherrima*) I

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The latex, stems, bracts, and flowers of *Euphorbia pulcherrima* Willd were chemically studied. Each part was extracted separately with petroleum ether. All the extracts contained germanicol, β-amyrin, and pseudotaraxasterol. From the latex was obtained a new sterol, $C_{24}H_{40}O$, pulcherrol. From the extracts of the stems were isolated an octaeicosanol and β-sitosterol.

In Mexico and Central America there are many native members of the *Euphorbiaceae*, particularly of the genus *Euphorbia* (which has over 1600 species) (1, 2), among them the *Euphorbia pulcherrima* Willd, called "flor de pascua" (Christmas flower). The bracts of this plant were used as galactopoietic and abortive agents by the Aztecs and they are still used for both purposes in medicine popular in some parts of Mexico (3, 4). Because of its colorful red bracts, the *E. pulcherrima* is actually cultivated in many parts of the world, being used as a flower arrangement at Christmastime, but there are only a few chemical studies of this plant. It has been reported that the ethanolic extracts of its petals have insecticidal properties and that its leaves contain alkaloids (5). Asen (6, 7) has identified by chromatographic and spectrophotometric methods four anthocyanins present in its bracts. The purpose of this work was to look for compounds with possible hormonal activity in different parts of the plant. From the petroleum extracts of the latex, bracts, and stems, β-amyrin, germanicol, and pseudotaraxasterol were isolated. In addition to these compounds, the latex provided a new steroid, $C_{24}H_{40}O$, for which the name, pulcherrol, is proposed. Also, in the extracts of the stems an octaeicosanol and β-sitosterol were identified.

EXPERIMENTAL

Melting points (uncorrected) were determined on a Kofler hot stage. Infrared spectra were taken on a Beckman IR-8 spectrophotometer and NMR spectra were obtained on a Varian A-60 instrument,[1] with tetramethylsilane as an internal standard and deuterochloroform as a solvent. Thin-layer chromatograms (TLC) were run on Silica Gel G (Merck), according to Stahl (8), and the system benzene–chloroform (7:1) (v/v) was used as solvent *A*.

Authentic specimens of triterpenoids were kindly supplied by Dr. Guy Ourisson and Dr. G. Poinsinet, Strasbourg University, and Dr. P. De Mayo, University of Western Ontario, Canada, whose assistance is acknowledged.

Microanalysis was by Dr. A. Bernhardt, Milheim/Ruhr, Germany. Identification methods and reactions were those described by Cheronis (9), and for the triterpenoids the methods used have been described by Steiner (10).

Plant Materials—The aerial part (stems, bracts, and flowers) and latex of *E. pulcherrima* were collected around Monterrey, Nuevo León, Mexico, in December 1963, 1964, and 1965.

Extraction of the Latex—The stems and terminal twigs were cut, and the flowing milky latex was collected. The latex (15 Gm.) was refluxed 12 hr. with methanol (150 ml.) and KOH (6 Gm.). The suspension was evaporated under reduced pressure, and the gummy residue was extracted five times with 50-ml. portions of ethyl ether. To the residue, water (100 ml.) was added, and a rubber-like material (9.8 Gm.) was obtained. The water solution was acidified with 5% HCl and an oily suspension (10.8 Gm.) was obtained; its principal component was palmitic acid which was isolated and identified by its physical constants and the melting point of its anilide (9). The combined ethyl ether solutions were washed with water and dried with Na_2SO_4. On TLC (solvent *A*) seven spots were detected with iodine vapors and also with 20% $SbCl_3$ in chloroform. The residue (2.8 Gm.), obtained on the removal of the ether, was dissolved in hot methanol–chloroform (20:1). On standing overnight, colorless crystals (1.45 Gm.) of crude pulcherrol were obtained, m.p. 110–116°. The mother liquors produced six spots on TLC; then the filtrate was evaporated and the residue (1.0 Gm.) was chromatographed on silica gel (150 Gm.). The substances were eluted successively by benzene, benzene–chloroform, and chloroform. A total of 36 fractions were collected; their composition was monitored by TLC. After rechromatography of several fractions and the corresponding recrystallization of the eluates, germanicol (23.5%), β-amyrin (9%), and pseudotaraxasterol (22%) were obtained.

Identification of the Known Substances (11)—Germanicol recrystallized from methanol, m.p. 178–180°; $[\alpha]_D^{24}$ +8°. [Reported (12) m.p. 180–181°; $[\alpha]_D^{24}$ +7°.] Acetylation with acetic acid anhydride in pyridine (12) produced an acetate, m.p. 276–280°; $[\alpha]_D^{24}$ +11°. [Reported (12) m.p. 279–280°; $[\alpha]_D^{24}$ +18°.] Mixed melting points with authentic germanicol and its acetate showed no depression.

β-Amyrin recrystallized from methanol, m.p. 197–199°; $[\alpha]_D^{24}$ +82°. [Reported (11) m.p. 199–200°; $[\alpha]_D^{24}$ +88°.] Acetylation with acetic acid anhydride in pyridine produced an acetate, m.p. 238–241°; $[\alpha]_D^{24}$ +81°. [Reported (11) m.p. 241°; $[\alpha]_D^{24}$ +81°.] Mixed melting point with an authentic β-amyrin showed no depression.

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[1] Spectra obtained by Dr. Roger Ketcham, School of Pharmacy, University of California, whose assistance is gratefully acknowledged.

Pseudotaraxasterol, recrystallized from ethanol, melted at 220–222°; $[\alpha]_D^{24}$ +46°. [Reported (11) m.p. 220–221°; $[\alpha]_D^{24}$ +50°.] Acetylation with acetic acid anhydride in pyridine (12) produced an acetate, m.p. 244–246°; $[\alpha]_D^{24}$ +50.4°. [Reported (11) m.p. 246–247°; $[\alpha]_D^{24}$ +53°.] Mixed melting point with an authentic pseudotaraxasterol showed no depression.

Purification of Pulcherrol—Pulcherrol was recrystallized from ethanol–chloroform and colorless needles, m.p. 115–116°, were obtained. It produced a single spot on TLC and gave positive results for the Liebermann-Burchard test for steroids and the tetranitromethane test for unsaturation. Negative results were obtained from the selenium dioxide–acetic acid test, for Δ^7 steroids (13); $[\alpha]_D^{24}$ (in $CHCl_3$) +35°. No absorption in the U.V.; infrared absorption: $\nu_{max.}^{CHCl_3}$ 3600, 2950, 2850, 1470, 1370, 1010 cm.$^{-1}$.

Anal.—Calcd. for $C_{24}H_{40}O$: C, 83.65; H, 11.70; O, 4.64. Found: C, 83.73; H, 11.59; O, 4.68.

The NMR spectrum: 5.2 (multiplet), 6.52 τ (singlet), 7.9–9.2 τ (38H, complex with five singlet signals).

Pulcherryl Acetate—Pulcherrol (100 mg.) was mixed with acetic anhydride (5 ml.) and pyridine (1.0 ml.). After it stood at room temperature for 12 hr., the mixture was diluted with water. The crude acetate was collected and recrystallized several times from methanol. The bright plates melted at 228–229°. I.R. spectrum: $\nu_{max.}^{CHCl_3}$ 2950, 2850, 1720, 1465, 1455, 1370, 1250, 1200, 1100, 1020, 980 cm.$^{-1}$. The NMR spectrum: 2.5 τ (multiplet) 7.9 τ (singlet, 3H), 3.1–9.2 τ (38H complex).

Anal.—Calcd. for $C_{26}H_{42}O_2$: C, 80.77; H, 10.95; O, 8.28. Found: C, 80.66; H, 10.74.

Oxidation of Pulcherrol—Pulcherrol (100 mg.) in pyridine (1 ml.) was mixed with 4 ml. of chromic acid–pyridine–acetic acid complex (14), and the temperature was held at 0° for 1 hr. Methanol (2 ml.) was added, then the mixture was diluted with water, and the ketone was recovered as usual. On recrystallization from methanol, needles (80 mg.), m.p. 151–152°, were obtained. I.R. spectrum: $\nu_{max.}^{CHCl_3}$ 2950, 2850, 1700, 1465, 1460, 1380, 1110, 1005 cm.$^{-1}$.

Anal.—Calcd. for $C_{24}H_{38}O$: C, 84.15; H, 11.18; O, 4.67. Found: C, 84.33; H, 11.28.

Epoxide of the Pulcherryl Acetate—A chloroform solution of pulcherryl acetate (50 mg.) was mixed with 50 mg. of *m*-chloroperbenzoic acid and 5 ml. of chloroform. The mixture stood at room temperature for 12 hr. Then, 5% NaOH solution (25 ml.) was added. The chloroform layer was rinsed with water, dried with Na_2SO_4, and evaporated. The residue was recrystallized from methanol–chloroform, m.p. 267–269°.

Anal.—Calcd. for $C_{24}H_{40}O_2$: C, 79.94; H, 11.19; O, 8.88. Found: C, 80.07; H, 11.26; O, 8.69.

Extraction of Stems—Dried stems (1400 Gm.) of *E. pulcherrima* were extracted in a Soxhlet apparatus with petroleum ether (b.p. 30–60°) for 100 hr. The green solution was evaporated and the greenish residue (7.0 Gm., yield 0.5%) was saponified as described for the latex, but the unsaponifiable material was extracted with several portions of chloroform. The chloroform solutions were mixed and added to a column of alumina (Merck). The elutions were obtained in the usual way (10) with benzene, chloroform, ethyl acetate, and ethanol. From the first fraction was obtained an aliphatic alcohol, 326 mg., which after recrystallization from chloroform–methanol melted at 63–65°.

Anal.—Calcd. for $C_{28}H_{58}O$: C, 81.87; H, 14.23; O, 3.90. Found: C, 81.53; H, 14.12; O, 3.86.

Also there were eluted and identified, as mentioned for the latex, germanicol, 370 mg., m.p. 177–179°; β-amyrin, 825 mg., m.p. 198–200°; pseudotaraxasterol, 263 mg., m.p. 237–240°; and β-sitosterol, 108 mg., m.p. 134–136°; $[\alpha]_D^{CHCl_3}$ −37°, from which β-sitosteryl acetate was produced by acetylation, in the usual manner (11); it melted at m.p. 125–127°. There was no depression of the melting point when each compound was mixed with an authentic specimen.

Extraction of Bracts and Flowers—Dried flowers and bracts (1700 Gm.) were extracted with petroleum ether (b.p. 30–60°) in a Soxhlet apparatus during 160 hr. The solution, containing 22.85 Gm. of solids, and showing five spots on TLC, was chromatographed on alumina (Merck). The fractions were eluted by benzene, chloroform, and ethanol. The crude ester mixtures, detected by I.R. spectroscopy on several fractions, were saponified with KOH in methanol as usual (10). The unsaponifiable material was extracted with ethyl ether and the solution was chromatographed on alumina. From the eluates there was obtained and identified germanicol (1.32 Gm.); β-amyrin (0.386 mg.); pseudotaraxasterol (649 mg.); the same aliphatic alcohol of 28 carbons isolated from the stems (64 mg.); and an aliphatic hydrocarbon, melting at 75–77°.

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