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LUPEOL AND 30-NORLUPAN-3β-OL-20-ONE FROM THE COATING OF THE CASTOR BEAN (*RICINUS COMMUNI* L.)

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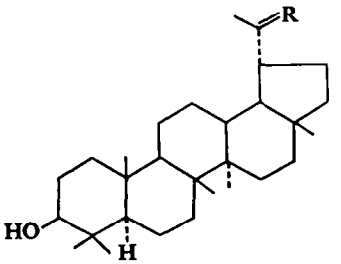
Abstract—Lupeol has been isolated and identified as the major constituent of the white crystalline coating of the *Ricinus communi* L. The isolation and identification of 30-Norlupan-3β-ol-20-one is also described.

THE white crystalline powder covering the larval cuticle of *Samia cynthia ricini* (Donovan) has been identified as triacontanol.[1] The castor bean (*Ricinus communi* L.) on which this silkworm feeds was also completely coated, except for its leaves, with a crystalline material. The two compounds therefore might be similar. However, the powder from the silkworm melted at 80°; that of the castor-bean plant melted at about 200°. A gas–liquid chromatographic (GLC) analysis of the material showed a major peak and several minor ones. Fractionation by column chromatography of the mixture afforded in 45 per cent yield a product with the analytical and spectral properties of an unsaturated alcohol, $C_{30}H_{50}O$, m.p. 212–214°, $[\alpha]_D$ +27°.

Acetylation with acetic anhydride and pyridine gave a crystalline monoacetate. The i.r. spectra of the alcohol and its acetate showed medium bands at 3075 and 1642 cm^{-1} and a strong band at 885 cm^{-1} that indicated a *gem*-disubstituted olefinic group.

The physical properties of the unsaturated alcohol and its acetate characterized this material to be lup-20(30)-en-3β-ol (I), commonly known as lupeol. Its identity was further confirmed by a direct comparison with an authentic sample of lupeol,[2] and its acetate by GLC, i.r. spectroscopy, and mixture melting points.

In addition to lupeol, the coating of the castor-bean plant yielded 12 per cent hydrocarbons, 5 per cent esters, and a hydroxy-ketone in a purified yield of 6·4 per cent.



I, R = CH_2
II, R = O

[1] W. S. BOWERS and M. J. THOMPSON, *J. Insect Physiol.* **11**, 1003 (1965).
[2] We thank Professor Sir E. Jones of Dyson Perrins Laboratory, Oxford, England, for this sample.

The mass spectrum of the hydroxy-ketone (m.p. 237–239°, $[\alpha]_D$ −10°) showed a strong parent ion with a molecular weight of 428·366 (confirmed by mass measurement). This value is consistent with the empirical formula $C_{29}H_{48}O_2$ (calculated 428·365; C = 12·0000). The most abundant fragment occurred at m/e 207, and it corresponded to a fragment observed to some extent in the spectra of all 3-hydroxy pentacyclic triterpenes.[3] Its mass spectrum also showed peaks at m/e 413, 410, and 385 indicating loss of Me, H_2O, and $COCH_3$, respectively. The nuclear magnetic resonance (NMR) spectrum of the hydroxy-ketone compound confirmed the presence of a methyl ketone group with a peak at 123 c/s and also showed six other methyl groups at 61, 59, 56, 49, 45, and 43 c/s, respectively.

The analytical values and spectral data suggested that the compound had the structure of a 30-norlupan-3β-ol-20-one (II). The physical properties also agreed closely with those reported[4] for synthetic II (m.p. 234–236°, $[\alpha]_D$ −14·6°). Identity was confirmed by a direct comparison of the i.r. and NMR spectra, the GLC behavior, and the mixture melting points with an authentic sample of II prepared from lupeol acetate.

As far as we know, 30-norlupan-3β-ol-20-one (II) has not been previously isolated from a plant source. Lupeol, however, is reported to be one of the more widely distributed of all triterpenes[5] and has recently been found in the blood of the silkworm[6] (*Bombyx mori* L.). Thus, the presence of lupeol in the coating of the castor-bean plant is not surprising; however, the large quantity is unusual.[7] Interestingly, though lupeol represented 45 per cent of the plant's coating, the common occurring plant sterols could not be detected by GLC; however, a chloroform–methanol extract of the plant showed that β-sitosterol and lupeol were present in equal quantities with stigmasterol, campesterol, and brassicasterol present in lesser amounts.

Whether the presence of lupeol (I) as the major constituent and of 30-norlupan-3β-ol-20-one (II) in the coating of the castor-bean plant have any functional significance is not known. However, it is tempting to speculate that one possible role for I may be that of an antifungal agent, though it may simply be an intermediate or end-product of a particular biogenetic scheme. The investigation of the role or roles of lupeol and of II in the coating of the castor-bean plant should be of considerable interest to plant biochemists.

EXPERIMENTAL

Melting points were taken on Kofler block and are corrected. Mention of a company name or a proprietary product does not necessarily imply endorsement by the U.S. Department of Agriculture. Rotations were determined in approximately 1 per cent solutions in $CHCl_3$ at 23°. I.r. spectra were obtained with a Perkin–Elmer Model 221 prism-grating spectrophotometer. NMR spectra were recorded at 60 Mc with a Varian A-60A NMR spectrometer by using CCl_4 as the solvent and TMS as an internal NMR standard. GLC analyses were made on Barber–Colman model 10 chromatograph and gas–liquid chromatographic systems used were 0·75 per cent SE-30 and 1 per cent QF-1. The mass spectra were measured by using a CEC-21-110B mass spectrometer, and the ionization energy was 70 eV.

Isolation of Lupeol (I) from the Coating of the Castor Bean Plant

A total of 1·0 g of the crystalline coating of the castor-bean plant obtained by wiping the covering of the plant with ether-extracted cotton moistened with benzene was chromatographed on 60 g of hexane–benzene (1:1)-washed neutral alumina (Woelm, activity grade II), and eluted as follows:

[3] H. BUDZIKIEWICZ, J. M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963).
[4] L. RUZICKA and G. ROSENKRANZ, *Helv. Chim. Acta* **23**, 1311 (1940).
[5] J. SIMONSEN and W. C. J. ROSS, *The Terpenes*, Vol. IV, p. 339. Cambridge University Press, Cambridge (1957).
[6] M. SUZUKI and N. IKEKAWA, *Chem. Pharm. Bull.* **14**, 1049 (1966).
[7] G. EGLINTON and R. J. HAMILTON, *Science* **156**, 1322 (1967).

Fraction	Solvent	Vol, ml	Wt, g
1	Hexane–benzene (1:1)	400	0·256
2	Benzene	400	0·371 (pure lupeol)
3	Ether	400	0·240
4	Chloroform	200	0·027
5	Chloroform–methanol (98:2)	200	0·032
6	Methanol	200	0·011

Fraction 2 contained pure lupeol, as indicated by m.p. and GLC analysis. Fraction 1, which still contained hydrocarbons, esters, and some lupeol was rechromatographed over 15 g of hexane-washed alumina, and the following 100-ml fractions were collected: Hexane, hexane–benzene (1:1), benzene, and ether. The hexane fraction yielded 120 mg of hydrocarbons, the hexane–benzene (1:1) fraction gave 50 mg of esters, and the benzene fraction gave 80 mg of pure lupeol. The ether fraction contained only a trace quantity of material. The fractions containing lupeol were combined and recrystallized from acetonitrile to give 400 mg of *lupeol* (*I*) as rods, m.p. 212–214°, $[\alpha]_D$ +27° $\nu_{max}^{CS_2}$ 3615 cm^{-1} (hydroxyl), 3075, 1642, and 885 cm^{-1} (*iso* propenyl group); lit.[8] m.p. 215°, $[\alpha]_D$ +27°.

The material gave no melting point depression with an authentic sample of lupeol, identical i.r. spectra, and relative retention times on 0·75 per cent SE-30 (temperature 236°, retention time 16·7 min, cholestane time 4·95 min). (Calc. for $C_{30}H_{50}O$: C, 84·44; H, 11·81. Found: C, 84·28; H, 11·82 per cent.)

The acetate of I (acetic anhydride–pyridine, 18 hr room temperature) was obtained as needles from acetone–methanol, m.p. 214–215°, $[\alpha]_D$ +35°; lit.[7] m.p. 218°, $[\alpha]_D$ +43°. (Calc. for $C_{32}H_{52}O_2$: C, 81·99; H, 11·18. Found: C, 82·26; H, 11·43 per cent.)

30-Norlupan-3β-ol-20-one (II) from the Coating of the Castor-Bean Plant

Gas–liquid chromatography on a 0·75 per cent SE-30 column of fraction 3 from the original column fractionation, which should have contained the phytosterols if any were present, showed one major peak with a retention time of 24 min (cholestane time 5·05 min) and two other components having a greater retention time. The common phytosterols will appear within 9 to 16 min under these conditions. The 240 mg of material from fraction 3 was rechromatographed over 15 g of benzene–hexane (6:1)-washed alumina, activity grade II, and the following 50-ml fractions were collected: 1–4, solvent benzene–hexane (6:1); 5–11, benzene–hexane (9:1); 12–15, benzene; and 16–20, ether. The fractions were monitored by GLC and the fractions (5–15) exhibiting only one component having a retention time of 24 min were combined and recrystallized from ether–hexane to give 64 mg of elongated needles, m.p. 237–239°, $[\alpha]_D$ −10°, ν_{max}^{Nujol} 1697 cm^{-1} (ketone) and 3505 and 3470 cm^{-1} (hydroxyl).

The NMR spectrum showed peaks at 123 c/s ($COCH_3$) and 61, 59, 56, 49, 45, and 43 c/s (six methyl groups). (Calc. for $C_{29}H_{48}O_2$: C, 81·24; H, 11·28. Found: C, 81·58; H, 11·08 per cent. Calc. M.W. 428·366. Found: 428·365 (mass measurement)); mass spectrum: m/e 428 (M^+ peak) other principal peaks at 413, 410, 385, 367, 328, 274, 207, 189, 135, 121, 95, 81, 55, and 43.

The material was identical by direct comparison with synthetic II by GLC, i.r. and NMR spectra and by mixture melting points.

Synthetic 30-Norlupan-3β-ol-20-one (II) from Lupeol Acetate

A mixture of 55 mg of lupeol acetate, 10 ml of dry ether, 80 mg of OsO_4, and 2 drops of pyridine was allowed to stand for 24 hr at room temperature. The mixture was concentrated *in vacuo*, and the residue was refluxed for 1 hr with 40 ml of 95 per cent ethanol, 20 ml of water and 1·0 g of Na_2SO_3. The solution was cooled, and the black precipitate was removed by filtering; then the filtrate was refluxed with 1·0 g of KOH for 1 hr. Most of the solvent was removed *in vacuo*, and the solution was diluted with water, and the precipitate was collected. The crude precipitate of the triol, m.p. 265–272°, and 130 mg of $NaIO_4$ in 5 ml of 95 per cent ethanol, 15 ml of dioxane, and 2 ml of water, was allowed to react for 18 hr at room temperature. The solution was diluted with water and extracted with ether. The ethereal solution was washed with water, dried (Na_2SO_4), and concentrated to dryness *in vacuo*. The residue was chromatographed over 10·0 g of benzene–hexane (3:1)-washed activity grade II alumina (Woelm), and the following 100-ml fractions were collected: One fraction of benzene–hexane (3:1), two fractions of benzene–hexane (6:1), and two fractions of ether. The first fraction of benzene–hexane (6:1) gave 5 mg of lupeol, and the first fraction of ether yielded 36 mg of II. One recrystallization from ether–hexane gave 30 mg of rods; m.p. 237–239°, $[\alpha]_D$ −11°; ν_{max}^{Nujol} 1697 cm^{-1} (ketone) and 3505 and 3470 cm^{-1} (hydroxyl); lit.[4] m.p. 234–236°, $[\alpha]_D$ −14·6°. The NMR spectrum of synthetic II showed peaks at 123 c/s ($COCH_3$) and at 61, 59, 56, 49, 45, and 43 c/s (six methyl groups).

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[8] D. H. R. Barton in *Chemistry of Carbon Compounds* (edited by E. H. Rodd), Vol. II, Part B, p. 729. Elsevier, New York, N.Y. (1953).