Betulinic Acid

The solid A was suspended in H_2O and by acidification and extraction with Et_2O crude betulinic acid (II) was obtained (215 mg) identified as described below. Methylation with diazomethane gave the ester, from MeOH, m.p. 209–210°, $[\alpha]_D^{18}$ +6·5 (*ca.* 1·00, $CHCl_3$). Acetylation of the ester with Ac_2O–Pyridine gave the acetate, from MeOH, m.p. 206–209°. It was identical (i.r., mixed m.p., TLC) to an authentic sample.

Extraction of Colletia paradoxa

The plant material was collected in Balcarce (Provincia de Buenos Aires) in February 1969. The extraction and isolation procedures were the same as described above. From 1·6 kg of ground roots 18 g of ethereal extracts were obtained.

Ceanothic Acid

From 2 g of the above residue 1·5 g of ceanothic acid were obtained, m.p. 339–341° (decom.) $[\alpha]_D^{18}$ +37·5 (*ca.* 0·85, $CHCl_3$) identical with that of an authentic sample of ceanothic acid (i.r., mixed m.p., TLC). From the mother liquors only several minor components were detected in silica gel plates.

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SOLANACEAE

LUPEOL IN TISSUE CULTURES OF *SOLANUM XANTHOCARPUM*

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Abstract—Triterpenes occurring in tissue cultures of *Solanum xanthocarpum* were investigated. The major component was identified as lupeol from TLC behaviour, i.r. spectra and m.p. of the parent compound and its derivative.

UTILIZATION of plant tissue cultures in the study of biosynthetic pathways of natural compounds is of considerable interest and a few such systems have proved useful for this purpose.[1–3] In the course of investigations on the biosynthesis of steroids present in *Solanum xanthocarpum* tissue cultures,[4,5] the occurrence of triterpenes in the callus was examined. This report concerns the isolation and identification of the major triterpene, lupeol.

EXPERIMENTAL

Four-week-old callus which had been under continuous subculture for the past 4 years and grown on Murashige and Skoog's basal medium with additives[6] was used as source material. 100 gm of oven-dried tissue was powdered and soxhlet extracted with chloroform for 24 hr. The extract was washed first with

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N NaOH and then with N HCl to remove the acidic and basic components. The chloroform layer, consisting of the neutral components, was further washed with water, dried over sodium sulphate and concentrated to dryness. The neutral fraction was chromatographed on silica gel (< 0·08 mm) column impregnated with 10% silver nitrate using light petroleum–chloroform gradient. Fractions containing the triterpenes were pooled and further separated by TLC using silica gel G impregnated with silver nitrate 10%, and the solvent system chloroform–petroleum ether (70:30). The major band on elution with chloroform gave 27 mg of a homogeneous product which on crystallization from aqueous alcohol gave 19 mg of pure compound (m.p. 215°). The i.r. spectrum of the compound showed strong absorption bands at 3400 cm^{-1} (OH) and 885 cm^{-1} ($C{=}CH_2$) and was super-imposable with the authentic sample of lupeol. An acetyl derivative of the compound was prepared using acetic anhydride and pyridine. On crystallization from methanol, it melted at 217–218° and showed no depression in melting point on admixture with authentic lupeol acetate. The mobility of the parent compound and its acetate on a TLC plate of silica gel 'G' was the same as lupeol and its acetate respectively in different solvent systems, thereby confirming the identity of the compound as lupeol.