Triterpenoids from the Leaves of *Ilex latifolia* Thunberg

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From the leaves of *Ilex latifolia* Thunberg, ursolic acid has been isolated by Kariyone et al.[1] and by Takemoto et al.[2] The present paper will deal with the isolation and identification of β-amyrin, lupeol, taraxerol, uvaol and β-sitosterol, besides that of ursolic acid, and with the isolation of an unknown triterpenoid acid, $C_{30}H_{48}O_3$, m. p. 296—298°C (decomp.), from the leaves of this plant. In this experiment, α-amyrin,[3] which has been isolated from the bird-lime of this plant, was not found. The structure of the triterpenoid acid is now being elucidated.

Experimental*

The Isolation and Identification of β-Amyrin, Lupeol, Teraxerol, Uvaol and β-Sitosterol.—Fresh leaves (6 kg.), collected in Kyoto Prefecture in August of 1963, were cut into chops and percolated with alcohol (55 l.) at room temperature for a week. The evaporation of the alcoholic extract afforded a tarry mass, which was then treated with water. A dark green, oily mass then separated, along with a greenish-white, amorphous material, from a turbid tan brown solution (a). The dark green, oily mass gave, after having been treated with a 50% alcoholic solution, a greenish-white amorphous material. The alcoholic filtrate was again evaporated to dryness and treated with water; the aqueous solution was then combined with the foregoing solution a. Both greenish-white, amorphous materials were continuously extracted with hexane. The unextracted amorphous material was found to be almost pure ursolic acid after purification through its potassium salt. The hexane solution was evaporated to dryness, and the residual resinous matter was divided into neutral and acidic fractions. From the acidic fraction, ursolic acid was isolated. When the crystallized neutral fraction was triturated with hexane and recrystallized from 80% alcohol, it afforded needles, m. p. 220—222°C; the identity of which with uvaol which had been synthesized by the lithium aluminium hydride reduction of methyl ursolate was estabished by a mixed melting point determination and an infrared comparison.

From the mother liquor of uvaol needles, m. p. 258—266°C, were separated. These were purified by silica-gel chromatography, giving needles, m. p. 272—274°C, which were found to be identical with taraxerol by a mixed melting point determination and an infrared comparison.

The mother liquor of taraxerol was mixed with silica gel and was fractionated by silica-gel chromatography, using a *n*-hexane - ethyl acetate mixture as the elution solvent.

Fractions showing R′ values of 0.80—0.82 on TLC were collected and recrystallized from alcohol or acetone to afford fine needles, m. p. 148—167°C. These were then acetylated with acetic anhydride and concentrated sulfuric acid, and the acetate mixture was fractionally recrystallized from ethyl acetate. The less soluble part of acetate in ethyl acetate gave flat rectangular plates, m. p. 293—294°C; undepressed after admixture with an authentic sample of taraxeryl acetate; its identity was confirmed by an infrared comparison.

The more soluble part gave, after tedious recrystallization from ethyl acetate, rods, m. p. 234—236°C, and floculent needles, m. p. 211—213°C. The former and its deacetylated alcohol (m. p. 195—196°C) were shown to be identical with β-amyrin acetate and β-amyrin, respectively, by a mixed melting point determination and an infrared comparison. The deacetylation of the latter gave microneedles, m. p. 210—211°C, from acetone. Its benzoate showed a m. p. of 263°C (from ethyl acetate). The identities of the alcohol and its benzoate were confirmed by a mixed melting point determination and by an infrared comparison with authentic samples of lupeol and its benzoate.

From the fractions showing an R_f value of 0.70, glistening leaflets, m. p. 135—136°C, undepressed after admixture with an authentic sample of β-sitosterol, were obtained.

Uvaol was also obtained from the fractions showing an R_f value of 0.50—0.53.

Some crystalline materials were obtained from the fractions showing lower R_f values, but they were not determined.

The Isolation of a Triterpenoid Acid, $C_{30}H_{48}O_3$.—The aqueous solution a was extracted with ether and, after the ether had been removed, was treated with a neutral lead acetate solution. The precipitated yel-

1) T. Kariyone, Y. Hashimoto and T. Kikuchi, *Yakugaku Zasshi*, **69**, 314 (1949).
2) T. Takemoto and K. Takahashi, *Yakugaku Kenkyu*, **22**, 301 (1952).
3) K. Yagishita, *Bull. Agr. Chem. Soc. Japan*, **21**, 157 (1957).

* The melting points are uncorrected. The microanalyses and the measurement of the infrared spectra were made by M. Goda and his associates of Osaka City University. Throughout this work the content and homogeneity of each material were checked by thin-layer chromatography (TLC) with a *n*-hexane: ethyl acetate=7 : 3 solvent.

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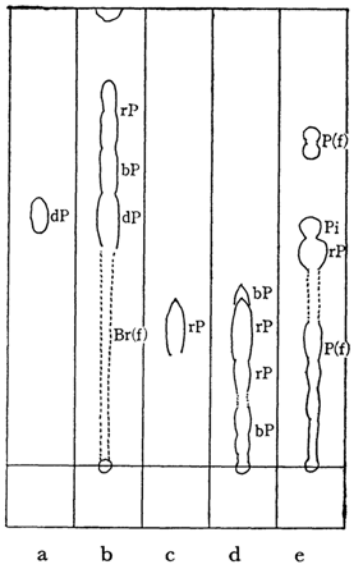

Fig. 1. Thin layer chromatogram of
a) a glucoside, m. p. 220—222°C
b) a glucoside mixture
c) a triterpenoid acid, m. p. 296—298°C
d) an acidic fraction of hydrolysate of b
e) a neutral fraction of hydrolysate of b.
Solvent: *n*-buthanol : acetic acid : water = 4:1:1 (a and b), *n*-hexane : ethyl acetate = 7 : 3 (c, d and e)
Development of color: 10% sulfuric acid
dP: dark purple, rP: reddish purple, bP: bluish purple, P: purple, Pi: pink, Br: brown, f: faint

lowish-white mud was dried and extracted repeatedly with boiling alcohol. The combined alcohol extracts were evaporated under reduced pressure to afford yellowish-white powder upon drying. This powder was crystallized from pyridine when small portions of water were added; then it was recrystallized from a diluted methanol or ethanol solution to give fine needles, m. p. 220—222°C. This compound afforded, on hydrolysis with 6 N hydrochloric acid in methanol, a triterpenoid acid, m. p. 296—298°C (decomp.); $[\alpha]_D^{12}$ +283° (*c* 1.03, chloroform : methanol = 1 : 1), whose R_f value on TLC was 0.32—0.33* (Fig. 1). Liebermann-Burchard reaction: purple; tetranitromethane test: yellow.

Found: C, 79.08, 78.94; H, 10.18, 10.07;. Calcd. for $C_{30}H_{48}O_3$: C, 78.90; H, 10.59%.

The acetylation of the acid with acetic anhydride and pyridine gave a reversible acetate, m. p. 262—264°C (decomp.).

Found: C, 77.12, 77.12; H, 9.66, 9.81. Calcd. for $C_{32}H_{50}O_4$: C, 77.06; H, 10.11%.

When the aqueous solution a (2 l.) stood after acidification with hydrochloric acid, there was precipitated a large amount of a reddish-tan resinous matter which solidified upon being dried (31 g.). This was found to be a mixture of glucosides containing the above-mentioned glucoside, m. p. 220—222°C; on hydrolysis with hydrochloric acid, it gave the above-mentioned triterpenoid acid and several neutral compounds (Fig. 1).

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* On the same TLC, ursolic acid showed an R_f value of 0.35—0.36 and oleanolic acid, one of 0.38.