468. *Triterpenoids. Part LI.* The Isolation and Characterisation of Glabric Acid, a New Triterpenoid Acid from Liquorice Root.*

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A new triterpenoid acid, $C_{30}H_{46}O_5$, which we name glabric acid, has been isolated from liquorice root. Glabric acid contains, in addition to a carboxyl group, two hydroxyl groups and an αβ-unsaturated ketone group, and is probably a hydroxy-18α-glycyrrhetic acid.

The glycyrrhetic acid used by us during a study of its stereochemistry [1] was isolated from liquorice (*Glycyrrhiza glabra*) root following a method described by Ruzicka and Leuenberger.[2] The isolation of the acid and its acetate was tedious and several modifications of the method were made in an attempt to facilitate the purification. In one of these, crude glycyrrhetic acid was acetylated, the product esterified with diazomethane, and the crude methyl ester acetate chromatographed on alumina. Many crystallisations of a number of fractions yielded pure methyl glycyrrhetate acetate. The more soluble material from these crystallisations was again chromatographed on alumina to give, in very small yield, a methyl ester diacetate, $C_{35}H_{52}O_7$, from which the parent dihydroxy-acid, $C_{30}H_{46}O_5$, which we name glabric acid, was obtained by alkaline hydrolysis. Glabric acid was further characterised by the preparation of its diacetate, $C_{34}H_{50}O_7$, and its methyl ester, $C_{31}H_{48}O_5$. The acid, its methyl ester, its diacetate, and the methyl ester diacetate all show the characteristic ultraviolet absorption of an αβ-unsaturated ketone, and do not give a colour with tetranitromethane. Glabric acid consequently contains one carboxyl group, one carbonyl group, two hydroxyl groups, and one double bond; it follows from this and its molecular formula, $C_{30}H_{46}O_5$, that it is pentacyclic.

Although the very small amount available has not permitted a detailed structural study, its close association with glycyrrhetic acid suggests that it is either a hydroxyglycyrrhetic acid or a hydroxy-18α-glycyrrhetic acid. We favour the latter relation for the following reasons. The ultraviolet absorption spectra of glycyrrhetic acid and its derivatives, on the one hand, and 18α-glycyrrhetic acid derivatives on the other, show small differences in the position of the principal maximum. In this respect, glabric acid resembles 18α-glycyrrhetic acid :

	Acid	Methyl ester	Acetate	Methyl ester acetate
Glycyrrhetic acid	2480	2480	2480	2480
18α-Glycyrrhetic acid	2420	2430	2440	2430
Glabric acid	2420	2420	2420	2420

Hydrolyses of methyl glycyrrhetate acetate, methyl 18α-glycyrrhetate acetate, and methyl glabrate diacetate by refluxing with 3% methanolic potassium hydroxide for 2 hr. gave 8, 83, and 44% of the parent acids respectively. The relatively small

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[1] Beaton and Spring, *J.*, 1955, 3126.

[2] Ruzicka and Leuenberger, *Helv. Chim. Acta*, 1936, **19**, 1402.

hindrance to hydrolysis of the methoxycarbonyl group in methyl glabrate diacetate when compared with methyl glycyrrhetate acetate is support for the view that the former compound is derived from 18α-glycyrrhetic acid. The fact that methyl 18α-glycyrrhetate acetate is hydrolysed to the parent acid more easily than is methyl glabrate diacetate may be due to a close proximity of the methoxycarbonyl and one of the acetate groups in the latter.

Although glabric acid is very probably a derivative of 18α-glycyrrhetic acid, it may be an artefact derived from its 18β-isomer during the mineral-acid hydrolysis of the mixed saponins from liquorice. In this connection, we have isolated 18α-glycyrrhetic acid (as its methyl ester acetate) from liquorice root by chromatography of the more soluble fractions obtained after separation of methyl glycyrrhetate acetate, and in our opinion its presence is to be ascribed to epimerisation of glycyrrhetic acid during the acid hydrolysis of glycyrrhizic acid.

As described in the Experimental section, β-sitosterol has also been isolated from liquorice root.

Experimental

Specific rotations were measured in $CHCl_3$ (unless stated otherwise) at room temperature. Ultraviolet absorption spectra were measured in EtOH. Grade-II alumina and light petroleum of b. p. 60—80° were used for chromatography.

Isolation of 18α-Glycyrrhetic Acid from Liquorice Root.—The crushed root (13·5 kg.) was boiled for 5 hr. with water (75 l.), the mixture filtered, and the solid again extracted with boiling water (75 l.) for 5 hr. The combined aqueous filtrates were concentrated to 10 l. and the cold extract was treated with concentrated sulphuric acid (625 c.c.) to give crude glycyrrhizic acid (*ca.* 2·7 kg.) which was washed with water. The crude glycoside (900 g.) was treated with boiling 3% sulphuric acid (4 l.) for 5 hr. The brown solid was collected, washed with water, dried in air at 80°, and powdered. The powder was extracted with boiling chloroform–ether (1 : 1; 4 l.), the extract evaporated, and the residue acetylated at 100° with pyridine and acetic anhydride for 1 hr. Cautious addition of water–methanol to the acetylated mixture precipitated a solid (filtrate A), many crystallisations of which from chloroform–methanol gave glycyrrhetic acid acetate (15 g.) as plates, m. p. 310—312°, $[\alpha]_D$ +145° (*c*, 1·1), $\lambda_{max.}$ 2480 (ε 11,200). The filtrate A was evaporated and a solution of the residue in chloroform (500 c.c.) treated with excess of diazomethane in ether. The crude methyl ester acetate was isolated in the usual way and purified by filtration of its solution in dry benzene through alumina. Removal of the benzene and many crystallisations of the residue from chloroform–methanol gave methyl glycyrrhetate acetate (1 g.) as plates, m. p. and mixed m. p. 299—301°. The combined chloroform–methanol mother-liquors from these crystallisations were evaporated and the residue (70 g.) in light petroleum–benzene (1 : 1; 2 l.) chromatographed on alumina (2 kg.). After the removal of fractions eluted with light petroleum–benzene mixtures and benzene, ether eluted fractions (total 9·6 g.) which crystallised from chloroform–methanol. Many recrystallisations from the same solvent yielded methyl 18α-glycyrrhetate acetate (460 mg.) as plates, identified by m. p. (254—256°) and mixed m. p. (254—256°), specific rotation {$[\alpha]_D$ +87° (*c*, 2·1)}, and ultraviolet absorption spectrum ($\lambda_{max.}$ 2430 Å, ε 11,600) (Found: C, 75·2; H, 9·7; OMe, 5·9. Calc. for $C_{32}H_{47}O_4$·OMe: C, 75·2; H, 9·6; OMe, 6·0%).

β-Sitosterol from Liquorice Root.—The crushed root (1150 g.) was extracted with boiling methanol (2 × 3 l.), and the filtered extract evaporated to a gum (250 g.). This was saponified by refluxing for 4 hr. with 5% aqueous-methanolic potassium hydroxide. The non-saponifiable fraction (6·5 g.) was isolated in the usual manner and acetylated at room temperature with acetic anhydride and pyridine for 3 days. A solution of the dry acetylated product (7·0 g.) in light petroleum (500 c.c.) was chromatographed on alumina (200 g.). Benzene–light petroleum mixtures eluted crystalline fractions (770 mg.), recrystallisation of which from chloroform–methanol gave β-sitosteryl acetate (240 mg.) as plates, m. p. and mixed m. p. 126—128°, $[\alpha]_D$ −39° (*c*, 1·1). Elution of the column with ether–methanol gave crystalline fractions (810 mg.) which yielded β-sitosterol (210 mg.) as blades (from chloroform–methanol), m. p. and mixed m. p. 139—141°, $[\alpha]_D$ −37° (*c*, 1·3).

Methyl Glabrate Diacetate.—Crude glycyrrhizic acid (900 g.) obtained as described above was kept at 100° with concentrated hydrochloric acid (1 l.) for 2 hr. After cooling, the solid was collected, washed with water, and dried. Acetylation of this solid with pyridine (200 c.c.) and

acetic anhydride (100 c.c.) gave an acetate mixture which was esterified by treatment in chloroform with an excess of diazomethane. The crude acetate methyl ester mixture (140 g.) in benzene (1·5 l.) was chromatographed on alumina (2·5 kg.). Elution with benzene (9 × 1 l.) gave fractions (86 g.) which crystallised from chloroform–methanol. The crystalline crops from each fraction were collected, combined, and recrystallised from chloroform–methanol, to give methyl glycyrrhetate acetate (16·2 g.) as plates, m. p. 300—301°, $[\alpha]_D$ +147° (*c*, 1·3), $\lambda_{max.}$ 2480 Å (ε 11,200). The combined chloroform–methanol mother-liquors from these crystallisations were evaporated and the residue (66 g.) in light petroleum (3 l.) was chromatographed on alumina (1·5 kg.). After elution with light petroleum (11 l.), and light petroleum–benzene mixtures (42 l.), benzene (10 l.) eluted a fraction (10·2 g.) which was repeatedly crystallised from chloroform–methanol, to give *methyl glabrate diacetate* (250 mg.) as plates m. p. 319—323°, $[\alpha]_D$ +42·5°, +43° (*c*, 2·3; 1·7), $\lambda_{max.}$ 2420 Å (ε 12,300) (Found : C, 71·7, 71·7; H, 9·1, 9·0; OMe, 5·7. $C_{34}H_{49}O_6$·OMe requires C, 71·9; H, 9·0; OMe, 5·3%). The ester does not give a colour with tetranitromethane in chloroform. Continued crystallisation of the methyl ester diacetate did not change the m. p. or $[\alpha]_D$. Continued elution of the column with benzene and with ether gave crystalline fractions (14 g.), many recrystallisations of which failed to give a homogeneous product.

Methyl Glabrate.—A solution of methyl glabrate diacetate (103 mg.) in 3% methanolic potassium hydroxide (50 c.c.) was refluxed for 2 hr. The solution was cooled, diluted with water, and separated into acid (40 mg.) and neutral (52 mg.) fractions. The latter crystallised from acetone–light petroleum, to give *methyl glabrate* as needles, m. p. 277—280°, $[\alpha]_D$ +16° (*c*, 1·3), $\lambda_{max.}$ 2420 Å (ε 12,600) (Found : C, 73·9; H, 9·7. $C_{31}H_{48}O_5$ requires C, 74·4; H, 9·7%). Treatment of methyl glabrate with acetic anhydride and pyridine at 90° for 30 min. regenerated methyl glabrate diacetate, m. p. and mixed m. p. 315—320°.

Glabric Acid.—The acid fraction (above) was crystallised from methanol and then from acetone–light petroleum, to yield *glabric acid*, m. p. 329—333°, $[\alpha]_D$ −26° (*c*, 1·0 in pyridine), $\lambda_{max.}$ 2420 Å (ε 11,000) (Found : C, 73·8; H, 9·6. $C_{30}H_{46}O_5$ requires C, 74·0; H, 9·5%).

Glabric acid diacetate was prepared from glabric acid by treatment with acetic anhydride and pyridine at 90° for 30 min. The acid diacetate separates from aqueous methanol as plates, m. p. 308—309° (decomp.), $[\alpha]_D$ +38° (*c*, 0·9), $\lambda_{max.}$ 2420 Å (ε 12,800) (Found : C, 71·5; H, 8·7. $C_{34}H_{50}O_7$ requires C, 71·55; H, 8·8%).

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