GYPSOGENIC ACID FROM THE ROOTS OF ACANTHOPHYLLUM SUBGLABRUM AND A. GYPSOPHILOIDES

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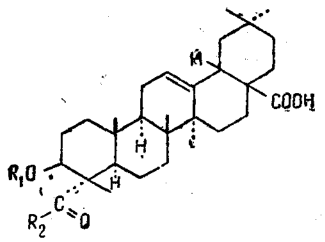
On hydrolyzing a methanolic extract of the roots of A. subglabrum Schischk. with 3% hydrochloric acid we obtained an amorphous mass of triterpene acids giving a positive Liebermann-Burchard reaction. It was impossible to separate the bulk of the acids into individual substances but by their acetylation a crystalline acetate with mp 345°–347° C (decomp.) was prepared from which a hydroxy acid, and then its methyl ester and the acetate of the methyl ester, were obtained after alkaline hydrolysis.

Compound	Mp, °C, according to			
	Jacobs [1]	Wedekind [2]	Ruzicka [3]	this paper
Acid $C_{30}H_{46}O_5$	—	364	380	above 370
Acetate $C_{32}H_{48}O_6$	—	321	325	345—347
Dimethyl ester $C_{32}H_{50}O_5$	244—246	244—245	249—250	244.5—246
Acetate of the dimethyl ester $C_{34}H_{52}O_6$	—	171	179—180	169.5—171

This acid had not previously been found in nature but proved to be identical with the gypsogenic acid (I) obtained by the oxidation of gypsogenin [2, 3] and of hederagenin, and also described under the names hederagic [1] and githagonolic acids [2]. The table gives the results of a comparison of the melting points of the acid and its derivatives with literature data.

To identify the gypsogenic acid more reliably, we synthesized its acetate from gypsogenin (II). Gypsogenic acid has also been isolated in considerably lower yield by the saponification of a methanolic extract of the roots of A. gypsophiloides Rgl. The saponin of this plant has been investigated by Abubakirov et al. [4]. Under mild conditions of acid hydrolysis similar to ours, they isolated vaccaroside (III). The latter was hydrolyzed, forming gypsogenin (II) under very severe conditions. The difference between the results obtained by Abubakirov [4] and our results is evidently due to a difference in the methods used for isolating the substances.



I $R_1 = H$ $R_2 = OH$
II $R_1 = R_2 = H$
III $R_1 = C_6H_9O_7$ (a D-glucuronic acid residue) $R_2 = H$

Experimental

The experiments were carried out with roots of A. subglabrum collected in the Turkmenian SSR in June 1963 and the roots of A. gypsophiloides obtained from the Alma-Ata Botanical Gardens. The melting points are uncorrected. The samples for analysis were dried at 100° C/10 mm for 20 hr.

Triterpenes from the roots of A. subglabrum. 1.5 kg of the dry ground roots previously extracted with chloroform were exhaustively extracted with methanol. The extract was evaporated to 1 *l* , 100 ml of concentrated hydrochloric acid was added, and the mixture was boiled for 4 hr. The reaction mixture was diluted with water, and the precipitate was filtered off, dried, and extracted with ether. The ethereal solution was treated with 1% aqueous alkali. A voluminous precipitate of salts deposited from the aqueous layer, and this was filtered off and dissolved in aqueous alcohol. The acids were precipitated by adding hydrochloric acid, and were dissolved in alcohol and purified by boiling with carbon. Evaporation yielded 8 g of a white amorphous mass.

Acetate of gypsogenic acid. A solution of 4 g of the amorphous acids in 30 ml of dry pyridine and 50 ml of acetic anhydride was left at room temperature for 40 hr. Then the mixture was treated in the usual way. Crystallization from chloroform gave small needles with mp 317°–321° C. After two recrystallizations from the same solvent the substance had mp 345°–347° C (decomp.), $[\alpha]_D$ +76° (c 0.92; dioxane). Yield 1.2 g.

Found, %: C 71.50, 71.40; H 9.17, 9.25. Calculated for $C_{32}H_{48}O_6 \cdot 0.5H_2O$, %: C 71.47; 9.18.

Gypsogenic acid. 0.5 g of the acetate was boiled in 80 ml of 3% alcoholic caustic potash for 2.5 hr. The solution was diluted with water to 150 ml and acidified with dilute hydrochloric acid. The acid was crystallized from aqueous methanol in the form of needles melting with decomposition above 370° C, $[\alpha]_D$ +90° (c 1.11; dioxane).

Found, %: C 72.62, 73.00, 72.79; H 9.59, 9.59, 9.54. Calculated for $C_{30}H_{46}O_5 \cdot 0.5\ H_2O$, %: C 72.69; H 9.56.

Dimethyl ester. 150 mg of the acid was treated with an ethereal solution of diazomethane obtained from 300 mg of nitrosomethylurea. The solution was kept at room temperature overnight and was then washed with alkali and with water. After the solvent had been distilled off, the residue was crystallized from acetone to form plates with mp 244.5°–246° C, $[\alpha]_D$ +84° (c 1.38; chloroform).

Found, %: C 74.45, 74.52; H 9.99, 9.73. Calculated for $C_{32}H_{50}O_5$, %: C 74.67; H 9.79.

Acetate of the dimethyl ester. A solution of 60 mg of the dimethyl ester in 5 ml of dry pyridine and 5 ml of acetic anhydride was kept at room temperature for 12 hr. The mixture was treated in the usual way. After crystallization from methanol, the substance melted at 169.5°–171° C.

Found, %: C 73.68; 73.76, H 9.56, 9.55. Calculated for $C_{34}H_{52}O_6$, %: C 73.61; H. 9.45.

Gypsogenic acid acetate from gypsogenin. A mixture of 0.5 g of gypsogenin, 5 ml of pyridine, and 10 ml of acetic anhydride was heated on a water bath for 3 hr. The mixture was decomposed with water, and the residue was filtered off and dissolved in 30 ml of acetic acid. The solution was treated, with stirring, with a mixture of 300 ml of sodium dichromate, 5 ml of water, and 2 ml of concentrated sulfuric acid. After 10 min, 2 ml of alcohol was added to the reaction mixture and the reaction product was precipitated with water. The precipitate was filtered off and recrystallized from chloroform, mp 345°–346° C (decomp.); $[\alpha]_D$ +79° (c 0.87; dioxane). A mixture of this substance and the gypsogenic acid acetate that we isolated from the plant melted at the same temperature.

Alkaline saponification of the acetate gave gypsogenic acid, which melted with decomposition above 370° C and gave no depression of the melting point in admixture with the acid isolated from the plant.

The triterpenes from the roots of A. gypsophiloides. The triterpenes were isolated by a method similar to that described. Two kilograms of the roots gave 18 g of amorphous acids.

The acetylation of 8 g of the acids under the conditions given above yielded 0.4 g of a crystalline substance with mp 344°–346° C (decomp.), which on saponification was converted into gypsogenic acid.

This gypsogenic acid and its acetate gave no depression of the melting points in admixture with the corresponding compounds isolated from *A. subglabrum.*

Summary

Gypsogenic acid, previously obtained by the oxidation of gypsogenin and hederagenin, has been found for the first time in *A. subglabrum* and *A. gypsophiloides.*

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