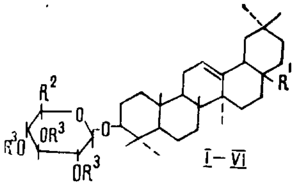
OLEANOLIC ACID 3-O-β-D-GLUCURONOPYRANOSIDE FROM THE ROOTS OF *Calendula officinalis*

L. P. Vecherko, É. P. Zinkevich,
and Leonid M. Kogan

UDC 547.918+547.597

From the roots of C. officinalis L. we have isolated by chromatography on a column of silica gel, in addition to the glycosides found previously [1-3], the more polar calenduloside E [1].



I–VI

I. $R^1 = R^2 = COOH$; $R^3 = H$.
II. $R^1 = COOH$; $R^2 = COOCH_3$; $R^3 = H$.
III. $R^1 = R^2 = COOCH_3$; $R^3 = H$.
IV. $R^1 = R^2 = CH_2OH$; $R^3 = H$.
V. $R^1 = R^2 = COOCH_3$; $R^3 = CH_3$.
VI. $R^1 = R^2 = CH_2OH$; $R^3 = CH_3$.

The glycoside (I), mp 278-280°C (decomp.) contained about 9% of incombustible residue and from its chromatographic behavior and IR spectrum was identified as oleanolic acid 3-O-β-D-glucuronoside, which has been isolated previously from C. officinalis [4] and a sample of which was kindly given to us by Dr. Kasprzyk (Poland). After desalting with a cation-exchange resin (Dowex 50 W×2, 100/200, H^+ form) or treatment with 2.5% H_2SO_4 at 100°C for 2 h, the glycoside (I) was obtained with mp 224-226°C (methanol), $[\alpha]_D^{20} + 14°$ (ethanol).

Found: mol. wt. 605 (spectrophotometrically) [5]. $C_{36}H_{56}O_9$. Calculated: mol. wt. 632.8.

IR spectrum: cm^{-1}: 1700, 1730 (COOH), 3450 (OH).

Literature data: mp 197-199°C (aqueous ethanol), $[\alpha]_D^{10} + 18.5 \pm 3°$ (ethanol) [4]; mp 210°C (decomp., acetone–hexane), $[\alpha]_D^{22} + 15.26°$ (methanol) [6]. On prolonged treatment of a methanolic solution of (I) with a cation-exchange resin or with 6% H_2SO_4 at 100°C for 7 h, partial methylation took place with the formation of oleanolic acid 3-O-(6-O-methyl-β-D-glucuronopyranoside) acid (II), with mp 202-204°C, $[\alpha]_D^{20} + 18.1°$ (methanol). IR spectrum, cm^{-1}: 1695 (COOH), 1745 (ester carbonyl), 3450 (OH).

Literature data: mp 177°C (aqueous methanol) [7].

The discrepancy between the melting points of (I) and (II) than those given in the literature made a stricter proof of the structure of the glycoside that we had isolated necessary.

The acetylation of (II) gave an acetate $C_{43}H_{66}O_{12}$ with mp 168-170°C (methanol), $[\alpha]_D^{20} + 34°$ (chloroform) containing, according to functional analysis and NMR spectroscopy (signal at 3.72 ppm), one methoxycarbonyl group.

The treatment of (I) and (II) with an ethereal solution of diazomethane gave a substance $C_{38}H_{60}O_9$ (III) with mp 240-242°C (methanol), $[\alpha]_D^{20} + 11.1°$ (chloroform). IR spectrum, cm^{-1}: 1720, 1740 (ester carbonyl), 3500 (OH). Functional analysis and NMR spectroscopy (signals at 3.56 and 3.72 ppm) confirmed the presence of two methoxycarbonyl groups.

All-Union Scientific-Research Institute of Medicinal Plants. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 560-561, July-August, 1973. Original article submitted January 31, 1973.

Literature data for the dimethyl ester of oleanolic acid 3-O-β-D-glucuronopyranoside: mp 201-208°C, $[\alpha]_D^{18} + 10.5 \pm 2°$ (chloroform) [4]; mp 201-204°C (methanol–ether), $[\alpha]_D^{27} + 15°$ (methanol) [8]; mp 200-205°C (decomp.), $[\alpha]_D^{20} + 11.5°$ (chloroform) [9].

The reduction of (II) with $LiAlH_4$ in dioxane formed the glycoside of erythrodiol (IV), $C_{36}H_{60}O_7$, mp 205-208°C, $[\alpha]_D^{20} + 28°$ (ethanol).

Literature data: mp 208-210°C, $[\alpha]_D^{20} + 31.5°$ (ethanol) [9]. The acid hydrolysis of (IV) gave glucose and erythrodiol, mp 235-237°C (acetone), $[\alpha]_D^{25} + 80°$ (chloroform).

Literature data: mp 236-237°C (acetone), $[\alpha]_D^{32} + 85.54°$ (c 1.23; chloroform) [10].

The methylation of (I) and (II) with methyl iodide [2] gave the permethylate $C_{41}H_{66}O_9$ (V) with mp 108-110°C, $[\alpha]_D^{20} + 36.3°$ (chloroform).

The hydrochloric acid cleavage of (V) gave methyl oleanolate and methyl 2,3,4-tri-O-methyl-D-glucuronate (identified by PC, TLC, and GLC), which confirms the presence of a $1 \rightarrow 3$ bond of the glucuronic acid with the genin. The reduction of (V) with $LiAlH_4$ in dioxane gave (VI), mp 140-142°C, $[\alpha]_D^{20} + 21.5°$ (methanol). When (VI) was hydrolyzed, erythrodiol and 2,3,4-tri-O-methyl-D-glucopyranose were found in the hydrolyzate (PC, TLC, and GLC), which shows the presence of a pyranose ring in the glucuronic acid in the glycoside (I).

The difficulty of acid hydrolysis and the lower specific rotation of (III) ($[\alpha]_D^{20} + 11.1°$) than of methyl oleanolate ($[\alpha]_D^{20} + 75°$) [11] shows the β configuration of the glucosiduronic bond. This is also confirmed by the difference in the molecular rotations of erythrodiol and its glycoside (IV).

Thus, the results obtained confirm the structure of the calenduloside E (I) that we isolated as oleanolic acid 3-O-β-D-glucuronopyranoside:

LITERATURE CITED

1. L. P. Vecherko, É. P. Zinkevich, N. I. Libizov, and A. I. Ban'kovskii, Khim. Prirodn. Soedin., 58 (1969).
2. É. P. Zinkevich, N. I. Libizov, and A. I. Ban'kovskii, Khim. Prirodn. Soedin., 22 (1971).
3. L. P. Vecherko, V. S. Kabanov, and É. P. Zinkevich, Khim. Prirodn. Soedin., 533 (1971).
4. S. Kasprzyk and Z. Wojcichowski, Phytochem., 6, 69 (1967).
5. G. L. Genkina, E. S. Kondratenko, L. G. Mzhel'skaya, and N. K. Abubakrov, Khim. Prirodn. Soedin., 501 (1965).
6. N. Kondo, V. Marumoto, and J. Shoji, Chem. Pharm. Bull., 19, 6, 1103 (1971).
7. Z. Wojcichowski, A. Jelonkiewicz-Konador, M. Tomaszewski, J. Jankowski, and Z. Kasprzyk, Phytochem., 10, 1121 (1971).
8. V. Hariharan and S. Rangaswami, Phytochem., 9, 2, 409 (1970).
9. N. K. Kochetkov, A. Ya. Khorlin, and V. E. Vas'kovskii, Izv. Akad. Nauk SSSR, Otd. Khim. Nauk, 1409 (1963).
10. N. Kondo, J. Shoji, N. Nagumo, and N. Komatsu, J. Pharm. Soc., Japan, 89, 6, 1852 (1960).
11. O. Jeger, Angew. Chemie, 63, 196 (1951).