UDC 547.972

# NEW FLAVANONE COMPOUNDS FROM EUPHORBIA STEPPOSA

O. M. Sotnikova, R. K. Chagovets, and V. I. Litvinenko

Khimiya Prirodnykh Soedinenii, Vol. 4, No. 2, pp. 82-87, 1968

In the study of plants of the family Euphorbiaceae, hitherto the main attention has been devoted to the triterpene compounds [1] and some other groups of substances. The phenolic compounds have been studied comparatively little, although, according to our preliminary results, this family contains them in considerable amount [2]. Continuing our investigation of the phenolic compounds of plants of the genus Euphorbia, we have found in the herb Euphorbia stepposa flavanone derivatives with a positive qualitative reaction with alkali and with sodium borohydride and hydrochloric acid [3].

The combined flavonoids, previously freed from water-soluble impurities, were separated on a polyamide column. Two substances of flavonoid nature were isolated. The first of them was an aglycone, and we have called it steppogenin. It gives positive reactions for flavanones [3], a red-brown coloration with ferric chloride, and a yellow coloration with zirconyl nitrate. A comparison of its properties with a number of known flavanone and flavanonol compounds [4] permit the assumption that this substance is probably new. The IR spectrum of steppogenin has a band at 1650 $cm^{-1}$ indicating the carbonyl group of a $\gamma$-pyrone. Bands with frequencies of 800 and 870 $cm^{-1}$ make it possible to assign steppogenin to the flavonoids of the phloroglucinol type [5], and bands in the region between 680 and 870 $cm^{-1}$ indicate that ring B must be 1,2,4-substituted [6].

The UV spectrum of steppogenin (see figure, curve 1) has two strong maxima in the short-wave region (at 228 and 288 m$\mu$) and an ill-defined maximum in the long-wave band (at 330 m$\mu$). The ratio of the intensities of the maxima of the short-wave and long-wave bands ($E_{330}:E_{288}$) is 11%, which, according to Wagner [6], determines its flavanone or flavanonol nature. Under the influence of sodium acetate, maxima at 230 and 330 m$\mu$ appear in the UV spectrum of steppogenin, the latter being stronger than the maximum at 288 m$\mu$ in the spectrum of the initial compound. Apparently, ionization of a 7-hydroxy group leads to a bathochromic shift of the maximum at 288 m$\mu$ by 42 m$\mu$. Ionization with sodium methoxide leads to a similar bathochromic shift and, moreover, in this case, an additional maximum appears at 245 m$\mu$. The complex-forming reaction with zirconyl nitrate causes a bathochromic shift of the maximum of the long-wave band by 45 m$\mu$. Thus, from the data of UV spectroscopy, it may be assumed that steppogenin contains free 5- and 7-hydroxy groups.

From the results of IR spectroscopy, it may be assumed that ring B has two other substituents, probably in the 3' and 4' positions. However, the weak degree of conjugation of ring B with the remaining part of the molecule in the compound under study does not permit a more definite conclusion to be drawn.

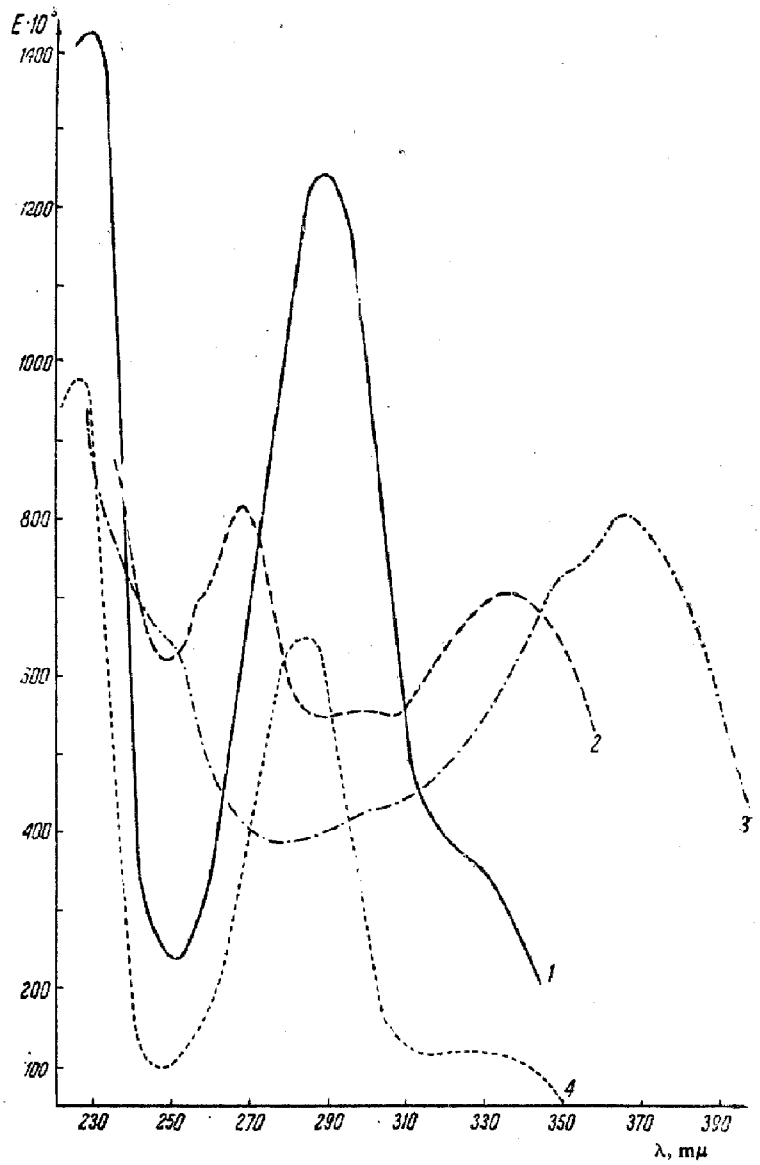
From the number of acetyl groups in the acetyl derivative of the genin four free hydroxy groups were found. Consequently, in view of the known properties of the flavanones, we oxidized the genin with iodine [7] to a flavone and obtained its IR spectrum (see figure, curve 2). The short-wave part of the spectrum of the flavone had a shoulder at 257 m$\mu$ and a maximum at 268 m$\mu$, and the long-wave part a maximum at 335 m$\mu$. As is well known, in flavones 3',4'-disubstituted with donor groups the short-wave region of the UV spectrum generally contains a maximum and a shoulder. In the UV spectrum of the flavone under consideration, this order is reversed. Sodium ethoxide causes a bathochromic shift of the long-wave maximum by 64 m$\mu$ with a considerable increase in its intensity, thus showing the presence of a free 4'-hydroxy group. Boric acid in the presence of sodium acetate causes no changes in the spectrum, and therefore the second substituent in ring B is not a free hydroxy group or else this group is not in position 3' as was to be expected from the results of IR spectroscopy.

To explain the features of the structure of ring B, we subjected steppogenin to alkaline cleavage, detecting in the resulting products phloroglucinol and $\beta$-resorcylic acid, identical with the acid isolated from the products of the alkaline degradation of morin (3,5,7,2',4'-pentahydroxyflavone). The formation of $\beta$-resorcylic acid from steppogenin shows that the second hydroxy group in ring B is in position 2'.

The red-violet coloration given by steppogenin with sodium borohydride and hydrochloric acid, and its spectral properties, establish the flavanone nature of this compound. Natural flavanones usually have a negative optical rotation, with the exception of (+)-sakuranetin [4]. Sapogenin, with a specific rotation of +8.5° must be regarded as another exception. On the other hand, most of the natural flavanonols have a positive rotation, and only in one case is it negative [4]. Consequently, assuming that steppogenin might be a flavanonol we performed some qualitative reactions for this class of compounds. It was found that steppogenin does not form a potassium salt at the 3-hydroxy group on reaction with potassium acetate, and in the presence of alkalies it isomerizes to a chalcone, like the flavanones. The UV spectrum of the chalcone from steppogenin has maxima at 250 and 365 m$\mu$ (see figure, curve 3). The ratio of the intensities of these maxima (161%) confirms the chalcone nature of the steppogenin isomer [6]. Thus, on the basis of our chemical and

spectral study, steppogenin can be characterized as (+)-5, 7, 2', 4'-tetrahydroxyflavanone, and its flavone isomer can be identified with lotoflavin, first isolated from the Arabian deervetch in the form of the glycoside lotusin [8], although the structure of this flavone is still disputable [9].

The second flavonoid compound isolated from the herb Euphorbia stepposa is, according to its qualitative reactions, also a flavanone. Acid cleavage of this substance leads to the isolation of an aglycone identical with steppogenin and D-glucose. The UV spectrum of the glucoside has maxima at 245, 285, and 330 mμ (see figure, curve 4). The ratio of the specific intensities of the absorption maxima at 330 mμ of the glucoside and its aglycone, 0.58, shows that this compound contains one molecule of glucose [10]. Consequently, this substance, which we have called stepposide, is a monoglucoside. Sodium acetate does not cause the changes in the UV spectrum of stepposide that are observed when a free




UV absorption spectra of steppogenin (1), the hydroxyflavone from steppogenin (2), the hydroxychalcone from steppogenin (3), and stepposide (4).

7-hydroxy group is present. Consequently, it may be assumed that in this monoside the carbohydrate substituent is located in position 7. The enzymatic cleavage of stepposide with emulsin shows the presence of a β-glucosidic bond. This conclusion is confirmed by a polarimetric analysis (see table) showing that the glucose stepposide is present in the pyranose form and is attached to the aglycones by a β-linkage.

The new flavanone glycoside, stepposide, obtained from Euphorbia stepposa, can be characterized as steppogenin 7-β-D-glucopyranoside.

## Experimental

The chromatographic study was carried out on paper of the Volodarskii Leningrad mill of type B in the following systems of solvents (by volume): 1) 1-butanol–acetic acid–water (4:1:5) and 2) 15% acetic acid.

The UV spectra were taken on an SF-4A spectrophotometer and the IR spectra on a UR-10 spectrometer (in potassium bromide tablets). The optical rotations were measured on an SPU-E spectropolarimeter.

Isolation of steppogenin and stepposide. Four kilograms of the dried and comminuted herb Euphorbia stepposa was extracted with ethanol (4 × 20 *l*). The combined extracts were evaporated to dryness and the flavonoids were extracted with hot water (4 × 0.5 *l*). The aqueous extract was filtered and purified from water-soluble impurities on a polyamide column. The flavonoids were completely desorbed from Kapron [~ nylon] with 70% ethanol, and the eluates were evaporated to dryness. The residue was dissolved in a small volume of 97% ethanol and the solution was diluted with chloroform in a ratio of 1:9. The chloroform-ethanolic solution of the combined flavonoids was transferred to a column of polyamide (6 × 100 cm). Elution was carried out with mixtures of ethanol and chloroform with gradually increasing concentrations of ethanol. The first eluates contained steppogenin, which, after recrystallization from 50% ethanol, had the form of white prismatic crystals with mp 255–257° C, $[\alpha]_D^{20}$ +8.5° (c 0.129; methanol).

Found, %: C 62.72; 62.48; H 4.20; 4.12; mol. wt. 287.5 (Rast). Calculated for $C_{15}H_{12}O_6$, %: C 62.50; H 4.16; mol. wt. 288.0.

Stepposide was obtained from the eluates containing 20% of ethanol and was crystallized from 50% ethanol. This gave white needle-like crystals with mp 148–150° C, $[\alpha]_D^{20}$ −52.5° (c 1.12; methanol).

Found, %: C 55.95; 56.07; H 4.92; 4.79; mol. wt. 449.5 (UV spectrophotometry). Calculated for $C_{21}H_{22}O_{11}$, %: C 56.00; H 4.88; mol. wt. 450.0.

Polarimetric Analysis of Stepposide and Steppogenin

| Substance | *M* | $[\alpha]_D$ | $[M]_D$ | $K_\Phi$ | $[M]_D K_\Phi$ | $\Delta c$ |
|---|---|---|---|---|---|---|
| Steppogenin | 288.0 | + 8.5 | + 24.5 | 0.32* | + 8.0 | — |
| Stepposide | 450.0 | −52.5 | −236.0 | 0.57 | −135.0 | −143.0 |
| Phenyl β-D-glucopyranoside | 256.0 | −72.0 | −182.0 | 1.00 | −182.0 | −182.0 |

*$K_\Phi$ for steppogenin was obtained from the ratio with the molecular weight of phenol.

Acetylation of steppogenin. A solution of 0.05 g of steppogenin in 2 ml of acetic anhydride was mixed with 2 ml of pyridine and left at 20° C for 24 hr. The steppogenin acetate had mp 156–158° C (from 80% ethanol).

Found, %: C 60.56; 60.49; H 4.43; 4.37; $COCH_3$ 37.51; 37.80; mol. wt. 455.5 (Rast). Calculated for $C_{15}H_8O_6(COCH_3)_4$, %: C 60.52; H 4.38; $COCH_3$ 37.71; mol. wt. 456.0.

Alkaline cleavage of steppogenin. A solution of 0.05 g of the genin in 10 ml of 20% caustic potash was heated at 100° C for 3 hr. The cooled reaction mixture was acidified with 20% sulfuric acid to pH 4–5. The cleavage products were extracted with ether (5 × 5 ml), and the ethereal solutions were washed with water, dried, and evaporated. By paper chromatography, the reaction products were shown to contain phloroglucinol with $R_f$ 0.77 (1) and 0.66 (2) and β-resorcylic acid with $R_f$ 0.94 (1) and 0.55 (2). The phloroglucinol and β-resorcylic acid obtained by the alkaline degradation of morin were used as samples.

Oxidation of steppogenin with iodine to the flavone. A mixture of 0.05 g of steppogenin and 0.5 g of fused sodium acetate was dissolved in 5 ml of acetic anhydride with heating. To this solution was added 0.25 g of crystalline iodine, and heating was continued for another 15 min [7]. After this, the mixture was diluted with 100 ml of water containing 0.5 g of potassium iodide and was left at 18° C for 12 hr. The precipitate that had deposited was filtered off, dissolved in 20 ml of 96% ethanol, and decolorized with metabisulfite. The ethanolic solution was diluted with a fourfold amount of water and the precipitate was filtered off. The resulting product was dissolved in 10 ml of 10% caustic potash (with heating). The filtered solution was neutralized with 20% sulfuric acid to pH 5 and left in the refrigerator to crystallize. The crystalline deposit of flavone that deposited was purified by preparative paper chromatography in 15% acetic acid [$R_f$ 0.10 (1), 0.90 (2)]. On the chromatogram, the hydroxyflavone was detected in the form of a dark spot in filtered UV light before staining, and after staining with alkali it assumed a greenish-yellow coloration. The hydroxyflavone was studied spectroscopically in the UV region.

Isomerization of steppogenin to the chalcone. A solution of 0.01 g of the genin in 5 ml of 1% caustic potash solution was heated at 100° C for 30 min. After acidification, the mixture of isomerization products was separated by preparative paper chromatography in system 2. The chalcone isomer had $R_f$ 0.05 (2) and 0.93 (1), giving a dark brown spot

before staining and a light yellow spot after spraying with alkali. The hydroxychalcone was studied spectroscopically in the UV region.

Acid hydrolysis of stepposide. 0.1 g of the glycoside was dissolved in 10 ml of 50% ethanol containing 5% of sulfuric acid, and hydrolysis was carried out at 100° C for 5 hr. The cooled solution deposited 0.062 g of white prismatic crystals, corresponding to 62% of the weight of the glycoside. The aglycone had mp 255–257° C (from 50% ethanol) and gave no depression of the melting point in admixture with steppogenin. The carbohydrate component was identified as D-glucose by chromatography and the preparation of the osazone.

Enzymatic hydrolysis of stepposide. 0.05 g of stepposide was dissolved in 5 ml of 50% ethanol, and the solution was diluted with water to 10 ml and mixed with a solution of emulsin (0.01 g in 5 ml of water). Hydrolysis was carried out at 40° C for 24 hr. Steppogenin and glucose were isolated from the hydrolysis products.

Summary

From the herb Euphorbia stepposa Zoz. two new compounds—steppogenin and stepposide—have been isolated. It has been established that steppogenin is (+)-5, 7, 2', 4'-tetrahydroxyflavanone and stepposide is 5, 7, 2', 4'-tetrahydroxyflavanone 7-β-D-glucopyranoside.

REFERENCES

1. G. Ponsinet and G. Ourisson, Phytochemistry, 4, 813, 1965.
2. O. M. Sotnikova and R. K. Chagovets, Farm. Zh., 20, no. 3, 58, 1965.
3. E. Eigen, M. Blitz, and E. Gunsberg, Arch. Biochem. Biophys., 68, 501, 1957.
4. T. A. Geissman, ed., The Chemistry of Flavonoid Compounds, Pergamon Press, N. Y., 1962.
5. I. P. Kovalev and E. V. Titov, Infrared Spectra of Some Groups of Natural Compounds; Atlas of Spectra [in Russian], Khar'kov, 1966.
6. I. B. Pridham, ed., Methods in Polyphenols Chemistry, N.Y., 44, 1964.
7. H. Rösler, T. I. Mabry, M. F. Crammer, and J. Kagan, J. Org. Chem., 30, 4346, 1965.
8. W. R. Dunston and T. A. Henry, Chem. News, 84, 26, 1901.
9. M. L. Doporto, H. M. Galagher, I. B. Gowan, A. C. Hugher, E. M. Philbin, T. Swain, and T. S. Wheller, J. Chem. Soc., 4249, 1955.
10. V. I. Litvinenko, Rast. resursy, 2, no. 4, 53, 1966.

22 January 1967

Khar'kov Pharmaceutical Institute

Khar'kov Chemical and Pharmaceutical Scientific Research Institute