FLAVONOID PULICARPIN FROM PULICARIA SALVIIFOLIA AND ITS HYPOLIPIDEMIC ACTIVITY

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UDC 547.972

The new compound 3',6-dihydroxy-3,4',5,7-tetramethoxyflavone has been isolated from the epigeal part of Pulcaria salviifolia Bunge and has been called pulicarin. Its structure has been established on the basis of physicochemical and spectral parameters (UV, IR, and mass spectra and PMR). Its hypolipidemic activity has been studied.

Derivatives of 6-hydroxyflavone have been isolated previously from plants of the genus Pulicaria (tribe Inuleae, family Compositae) growing in various regions of the terrestrial glove (P. dysentirica [1, 2], P. arabica [3], P. crispa [4], and P. paludosa [5]).

We have investigated the epigeal part of Pulicaria salviifolia Bunge [6] collected in the Pap region of Namangan province (Uzbekistan) in the flowering phase. By the column chromatography of an alcoholic extract on silica gel, together with ditertepenoids of the clerodane type obtained previously [7], we isolated a new flavonol derivative with the composition $C_{19}H_{18}O_8$ (I), mp 180-181°C, and we have called it pulicarin.

Its UV spectrum [λ_{max} 258, 272, 351 nm ($\log \varepsilon$ 3.61, 3.53, 3.77, respectively)] was characteristic for flavonol derivatives substituted in the C_3 hydroxy group. IR spectrum: 1360, 1470, 1520, 1610, 1665, 3410 cm^{-1}. Mass spectrum: $(M^+) = 374$, $(M^+ - 1) = 373$, $(M^+ - 15) = 359$.

The spectral parameters of pulicarin and its molecular mass, determined from its mass spectrum, showed that it was a dihydroxytetramethoxyflavone. The ion $(M^+ - 1) = 373$ in the mass spectrum corresponded to an ion formed on the detachment of a proton from the 6-OH group [8].

Analysis of the UV spectra of pulicarin with the ionizing and complex-forming additives $AlCl_3$, $AlCl_3$ + HCl, $NaOCH_3$, CH_3COONa, and CH_3COONa + H_3BO_3 showed that there were no free hydroxy groups in the 3, 4', 5, and 7 positions of pulicarin, i.e., these groups were methylated [9].

In the PMR spectrum ($CDCl_3$, 100 MHz) signals characteristic for hydroxy-methoxy substituted flavones were observed. A multiplet (2 H) at 7.55 ppm corresponded to the H-2' and H-6' protons and a doublet (1 H) at 6.95 to the H-5' proton, while a singlet (1 H) at 6.42 ppm was characteristic for an H-8 proton, the signals of four methoxy groups in the form of four narrow singlets (3 H) were observed in the region from 3.75 to 3.85 ppm, and the signal of a hydroxy group, which disappeared in the spectrum of the acetate, was shown at 6.05 ppm in the form of a singlet (1 H).

The acetylation of pulicarin formed the diacetate $C_{23}H_{22}O_{10}$ (II), which was characterized spectrally.

The fusion of pulicarin with caustic soda led to the formation of isovanillic acid, which unambiguously determined the position of the methoxy and hydroxy groups in ring B.

The combination of facts given above permitted the assumption for pulicarin of the structure of 3',6-dihydroxy-3,4',5,7-tetramethoxyflavone (I):

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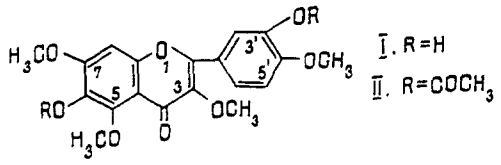
Translated from Khimiya Prirodnykh Soedinenii, Nos. 3,4, pp. 328-330, May-August, 1992. Original article submitted October 14, 1991.

0009-3130/92/0304-0286$12.50

TABLE 1. Influence of Pulicarin on the Level of Cholesterol in the Blood Serum of Normal Rats and of Animals with Hypolipidemias

Experimental conditions	Cholesterol in the blood serum, mg-%	Effect, %	p – the confidence level in relation to the intact animals
1. Intact animals (control)	86,3±3,3	—	—
2. Intact animals + pulicarin	64±3,9	−20	<0,001
3. Control (Triton WR 1339)	347±13,2	+400	—
4. Triton WR 1339 + pulicarin	280±18,4	−19	<0,05
5. Control (endogenous hyperlipidemia)	107±3,9	+24	—
6. Endogenous hyperlipidemia + pulicarin	89±6,2	−16	<0,05



I. R=H

II. R=COCH3

In view of the fact that in recent years a number of reports have appeared of a favorable influence of flavonoids on the organism in disturbances of the lipid metabolism [10], we have studied the action of pulicarin on the level of cholesterol and of diglycerides in the blood serum of normal rats and animals with endogenous hyperlipidemia. The results are given in Table 1.

Thus, the administration of pulicarin to normal animals over ten days led to a fall in the level of cholesterol in the blood serum by 20%.

The use of pulicarin under the conditions of a disturbed lipid metabolism led to its normalization. Thus, while the restoration of a Triton-induced hyperlipidemia was accompanied by a fourfold increase in the cholesterol level, the prior administration of pulicarin prevented an increase in its level by 19%. Its administration to animals with endogenous hyperlipidemia also led to a fall in the level of cholesterol in the blood (see Table 1).

EXPERIMENTAL

IR spectra were taken on a UR-20 spectrometer (tablets with KBr), PMR spectra on a Tesla BS-567 A instrument (δ scale, 0 – HMDS), mass spectra on an MKh-1310 instrument, and UV spectra on a Specord UV-VIS-75 spectrophotometer (ethanol). TLC control was conducted on Silufol plates (Chemapol) in the hexane–ethyl acetate (2:1) and chloroform–ethyl acetate (2:1) systems. The revealing agent was a 5% solution of vanillin in concentrated sulfuric acid.

Isolation of Pulicarin (I). The epigeal part of the plant with flowers, comminuted in the air, was extracted with chloroform, the chlorophylls were precipitated by the usual method, and the dried concentrated extract was chromatographed on a column of silica gel (100/160, Chemapol) with elution by hexane–ethyl acetate. The yield of pulicarin was 0.3% on the weight of the dry plant.

Acetylation of Pulicarin. Pulicarin was acetylated in the usual way with acetic anhydride in pyridine. This gave the diacetate (II), $C_{23}H_{22}O_{10}$, mp 191-192°C, M^+ = 458; IR: 1500, 1515, 1590, 1635, 1760 cm^{-1}.

Alkaline Fusion. A mixture of pulicarin and a 20% solution of potassium hydroxide was heated in the water bath for 8 h in a current of nitrogen. Then the reaction mixture was diluted with water and was acidified, and the reaction product was extracted with diethyl ether. Isovanillic acid, $C_8H_8O_4$, mp 249-250°C, was identified from its chromatographic mobility in the benzene–methanol–acetic acid (22.5:4:2) system in comparison with an authentic sample.

Biological Experiment. The hypolipidemic properties of the flavonoid were studied on random-bred male rats weighing 180-220 g. Hypolipidemia was brought about in the rats by

the intraperitoneal injection of Triton WR-1339 in a dose of 224 mg/kg, and endogenous hypolipidemia by the method generally adopted.

Pulicarin was used in a dose of 50 mg/kg. The preparation was injected into the intact animals in the course of 10 days. Under the conditions of hypercholesteremia, this was carried out twice: simultaneously with Triton WR-1339 and the generation of endogenous hypolipidemia and 2 h before the decapitation of the animals. The level of cholesterol in the blood serum was determined by the method of Abel et al. [11]. The results obtained were treated by the methods generally adopted.

LITERATURE CITED

1. K. E. Schulte, G. Rucker, and F. Muller, Arch. Pharm., 301, 115 (1968).
2. J. O. Pares, S. O. Oksuz, A. Ulubelen, and T. J. Mabry, Phytochemistry, 20, 2057 (1981).
3. I. El-Negoumy Sabry, M. A. Mansour Ragaa, and A. M. Salen Nabiel, Phytochemistry, 21, 953 (1982).
4. M. A. Yahya, A. M. Sayed, J. S. Mosso, J. F. Kozlowski, M. D. Antoun, M. Ferin, W. M. Baird, and J. M. Cassady, J. Nat. Prod., 51, 621 (1988).
5. A. S. Feliciano, M. Medarde, M. Gordaliza, E. Olmo, and J. M. Corral, Phytochemistry, 28, 2717 (1989).
6. Vascular Plants of the USSR [in Russian], Nauka, Leningrad (1981), p. 91.
7. M. R. Nurmukhamodova, Sh. Z. Kasymov, N. D. Abdullaev, G. P. Sidyakin, and M. R. Yagudaev, Khim. Prir. Soedin., 201 (1985).
8. J. H. Bowie and D. W. Cameron, Aust. J. Chem., 19, 1627 (1966).
9. F. J. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of Flavonoids, Springer, New York (1970).
10. M. T. Siddiqui and M. Siddiqui, Lipids, 11, 243 (1976).
11. L. L. Abel, B. B. Levy, and B. B. Bodie, J. Biol. Chem., 195, 357 (1952).

X-RAY STRUCTURAL INVESTIGATION OF GOSSYPOL AND ITS DERIVATIVES

XXII. STRUCTURE OF THE H-CLATHRATE OF GOSSYPOL WITH DIMETHYL SULFOXIDE

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UDC 547.737

In crystals obtained from solutions in DMSO, gossypol molecules are again present in the aldehyde tautomeric form. These crystals are H-clathrates with the channel type of structure which has much in common with the structure of the complexes of gossypol with methanol and with formic acid.

It is known that the gossypol molecule can theoretically exist in aldehyde, lactol, and quinoid tautomeric forms [1]. In all the clathrates (H-clathrates) and polymorphs of gossypol interpreted previously there is a single form – the aldehyde form [2-6]. In [7] on the basis of NMR studies it was reported that in samples obtained by recrystallization from benzene gossypol is present in the lactol form. However, the results of our x-ray structural investigations have refuted this statement [8]. In another paper [9], again on the basis of NMR investigations, it was established that in solutions of gossypol in DMSO there is a dynamic equilibrium between the lactol and aldehyde forms. The isolation of gossypol which, according to NMR results, existed in the form of the pure lactol tautomer has been reported relatively recently [10].

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Translated from Khimiya Prirodnykh Soedinenii, Nos. 3,4, pp. 330-334, May-August, 1992. Original article submitted July 9, 1991; revision submitted November 21, 1991.

0009-3130/92/0304-0288$12.50