6. V. V. Vandyshev, Yu. E. Sklyar, M. E. Perel'son, M. D. Moroz, and M. E. Pimenov, Khim. Prir. Soedin., 670 (1972).
7. V. V. Vandyshev, M. E. Perel'son, Yu. E. Sklyar, and M. D. Moroz, Khim. Prir. Soedin., 658 (1974).
8. V. V. Vandyshev, M. E. Perel'son, Yu. E. Sklyar, and M. D. Moroz, Khim. Prir. Soedin., 660 (1974).

ACETYLORIENTIN — A C-GLYCOSYLFLAVONE FROM *Hypericum hirsutum*

G. Kitanov, K. F. Blinova, and Kh. Akhtardzhiev

UDC 547.972

From the epigeal part of *Hypericum hirsutum* L. (hairy St. John's wort) we have isolated a number of flavonoid compounds, two of which have proven to be C-glycosylflavones [1]. There are no reports on the presence of flavones in species of the genus *Hypericum* L., with the exception of one on the chromatographic detection of luteolin in *H. hirsutum* [2]. Consequently, the compounds orientin and homoorientin that we have isolated are the first representatives of this type of flavonoid found in the genus *Hypericum*.

Continuing a study of the flavonoid complex of the herb hairy St. John's wort, we have isolated a flavonoid glycoside acylated with acetic acid (I).

From the results of qualitative reactions, fluorescence in UV light, and the Bryant test, it was assigned to the flavone glycosides [3, 4]. The intensity of the absorption $[E^{1\%}_{1cm}]$ = 393 characterized (I) as a monoside [5]. Analysis of the UV spectra showed four free hydroxy groups, in positions 3', 4', 5 and 7 [5, 6]. Acid treatment gave an intermediate product (II), which isomerized with the formation of a new substance having higher R_f values. No sugar and aglycone were detected as a result of this process. Alkaline hydrolysis gave only substance (II). These results show that the compound isolated is an acylated 8-C-glycosylflavone. The position of attachment of the carbohydrate moiety at C_8 was established on the basis of the chromatographic behavior of the isomers on acid hydrolysis and the presence of signals at 6.62, 1.75, and 1.95 ppm in the PMR spectrum of the full acetate of (I) (Fig. 1 and Table 1) which must be assigned to the H-6 protons and the 2"- and 6"-OAc groups, respectively (in acetate of 6-C-glycoflavonoids the signals of the H-8 proton and of 2"- and 6"-OAc groups are in the 7.25-7.40, 1.77-1.83, and 1.98-2.04 ppm regions [6-9]). The β configuration of the glycosidic bond was confirmed by the presence in the PMR spectrum of (I) of a doublet at 4.87 ppm with a spin–spin coupling constant J = 10 Hz [6-8].

The presence of an ester bond was confirmed by the IR and PMR spectra. The IR spectrum contained an absorption band at 1732 cm^{-1}. The PMR spectrum of compound (I) included a three-proton singlet at 1.74 ppm. In the IR and PMR spectra of (II) both these characteristics were absent. In all its indices (melting point, R_f values, UV, IR, and PMR spectra) the intermediate product (II) corresponded to orientin, which has been isolated from this plant.

The question of the nature of the acyl radical was solved on the basis of the PMR spectrum, a comparative analysis of the acetates of (I) and of orientin, and a chromatographic analysis of the hydrolysis products of the compound under investigation. The PMR spectrum lacked any signals of aromatic protons other than the signals of the flavone protons. In its melting point, a mixed melting point, its R_f values, and its IR and PMR spectra, the full acetate of (I) was identical with orientin octaacetate. When compound (I) was subject to acid and alkaline hydrolysis, no phenolic carboxylic acids were detected in the hydrolyzates. In order to confirm the presence of acetic acid in the molecule of (I) we obtained and identified its hydroxamate [10] and its diethylammonium salt [11-13]. In parallel, we performed the hydroxylaminolysis of ethyl acetate and obtained the diethylammonium salt of free acetic acid.

The problem of determining the position of attachment of the acetyl residue in the molecule was solved on the basis of the PMR spectrum of (I) and of its full acetate. The UV and

Leningrad Institute of Pharmaceutical Chemistry. Pharmaceutical Faculty of the Medical Academy, Sofia. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 154-158, March-April, 1979. Original article submitted November 14, 1978.

0009-3130/79/1502-0129$07.50

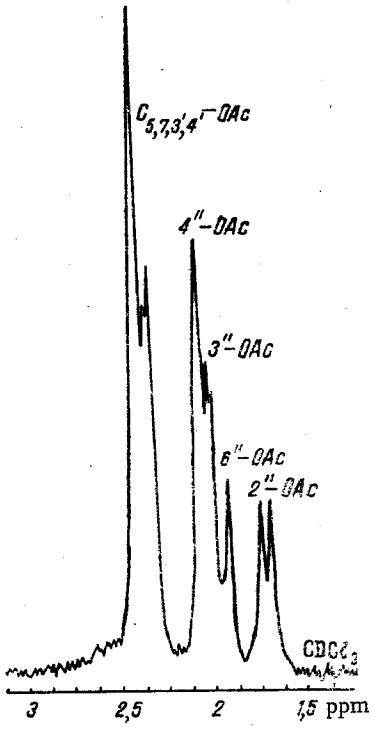


Fig. 1. Part of the PMR spectrum of the full acetate of (I) in deuterochloroform.

PMR spectra showed that the acetic acid was bound to a hydroxyl of the carbohydrate moiety. It is known from the literature that the signals of aliphatic acetyl protons in acetylated 8-C-glycosylflavones appear in clearly defined regions being very distinct from one another and from the signals of the acetyl groups of 6-C-glycosylflavones. At the same time, it has been found that the signal of a 2''-OAc group is present in a stronger field than the signals of all the other acetyl groups, being between 1.70 and 1.73 ppm. In the 6-C isomers, only the 2''-OAc signal is present in a discrete region and is located between 1.77 and 1.83 ppm [6-8]. The positions of attachment of the sugars and the fact that the acyl radicals are in the 2'' positions in a number of 6- and 8-C-biosides and acylglycoflavonoids have been established on the basis of these facts. Some literature information confirming our conclusions and also the results that we have obtained are given in Table 1.

As can be seen from Table 1, the acetyl group in (I) can be assigned only to the hydroxyl in position 2''. This made it possible for us to conclude that compound (I) is luteolin 8-C-(2''-O-acetyl-β-D-glucopyranoside) (2''-acetylorientin). It is a new natural compound, although a similar type of compound has been found by other authors [9, 14-16].

EXPERIMENTAL

The melting points were determined on a Kofler block. The spectra were obtained on the following instruments: Specord UV-VIS (UV) UR-20 in KBr tablets (IR); and Tesla BS 487C (in DMSO and $CHCl_3$, 80 MHz, δ, TMS) (PMR). Woelm polyamide was used as sorbent. Chromatographic monitoring was effected by PC in the following systems: 1) 15% acetic acid; 2) butan-1-ol–acetic acid–water (40:10:22); 3) ethanol–ammonia (9:1); 4) butan-1-ol-1.5 N ammonia (1:1) (upper layer); 5) butan-1-ol–diethylamine–water (50:0.5:7.5). TLC was performed on Merck type G silica gel in system 6): benzene-acetate (3:1).

Isolation. The air-dry herb hairy St. John's wort (1.5 kg) was extracted with 70% ethanol, the extracts were concentrated until the ethanol had been completely eliminated, and the aqueous residue was purified with chloroform. The flavonoids were extracted with ethyl acetate, the extracts were dried with anhydrous sodium sulfate, and the solvent was distilled off in vacuum to dryness. The mixture of polyphenols (50 g) was separated on polyamide sor-

TABLE 1. CS Values of the Signals of the —OAc Protons in the NMR Spectra of Fully Acetylated 8-C-Glycoflavones (in $CDCl_3$, δ, ppm, TMS)

Compound	Type of substitution				Literature source
	2″-OAc	3″-OAc	4″-OAc	6″-OAc	
On the basis of 9 acetylated compounds	1,70—1,73	1,98—2,02	2,08—2,10	1,90—1,95	[7]
2″-O-Glucosylvitexin acetate	—	7 aliphatic-OAc		(1,88—2,10)	[18]
2″-O-Glucosylorientin acetate	—	7 aliphatic-OAc		(1,89—2,12)	[18]
Vitexin acetate	1,72	2,01	2,09	1,91	[19]
2″-O-Xylosylvitexin acetate	—	1,99	2,08	1,92	[17, 19]
Orientin acetate	1,72	2,02	2,09	1,95	[19]
2″-O-Xylosylorientin (adonivernitol) acetate	—	1,97	2,07	1,92	[19]
2″-O-Acetyl-7-O-methylvitexin	1,53	—	—	—	[9]
2″-O-Acetyl-7-O-methylvitexin acetate	1,70	1,95	2,05	1,87	[9]
Obtained by ourselves					
Substance (I)	1,74	—	—	—	
Acetate of (I)	1,75*	2,05*	2,10	1,93	
Orientin acetate	1,75*	2,04*	2,10	1,93	

*Splitting. The splitting and broadening of the signals (see Fig. 1) is a characteristic for 2″-, 3″-, and 6″-O-Ac groups in the acetates of 8-C-glycosylflavones and is explained by the existence of two rotational conformers at temperature below 40°C [7, 8, 20].

bent in a gradient water—ethanol system. Substance (I) was eluted with 20% ethanol, and the deposited precipitate was recrystallized three times from 60% ethanol. After drying, 3.06 g of a light yellow crystalline powder was obtained.

2″-O-Acetylorientin (I). mp 236-237°C, R_f 0.53 (system 1) and 0.76 (system 2). UV spectrum, nm (log ε): λ_{max} (methanol) 259 (4.21), 271 (4.21), 350 (4.25); $[E_1^{1\%}{}_{cm}]$ = 393; + NaOMe: 272, 407; + $AlCl_3$:278, 427; + $AlCl_3$ + HCl:267 sh., 277, 300 d, 360, 380; + NaOAc:280, 387; + NaOAc + H_3BO_3:270, 378; IR spectrum (cm^{-1}); 3515-3370 (—OH); 1732 (ester bond); 1660 (C=O); 1618, 1520 (C=C).

PMR spectrum (DMSO, ppm, 25°C): 13,18, singlet, disappearing on deuterium exchange (C_5—OH); 7.57 (m, H-2′,6′); 6.90 (d, 8.5 Hz, H-5′); 6.70 (s, H-3); 6.28 (s, H-6); 4.87 (d, 10 Hz, H-1″); complex multiplet at 4.00-3.25 (protons of the carbohydrate residue); 1.74 (s, 2″-OAc).

The acid treatment of (I) for isomerization was performed with 10% H_2SO_4 and 2% HCl at 100°C for 3 and 4 h, respectively. To obtain substance (II), 45 mg of (I) was treated with 1% HCl on the boiling water bath for 1 h. After cooling, the yellow precipitate that had deposited was separated off, washed with water to neutrality, and dried at 100°C in vacuum.

Alkaline Hydrolysis. A solution of 10 mg of substance (I) in 2 ml of 0.5% KOH solution was kept at room temperature for 30 min. The saponification process was followed by paper chromatography. The mixture was neutralized with 1% HCl to pH 6, the solution was evaporated to small volume, and the precipitate that crystallized out was separated off and was washed with 30% ethanol and then with water. This gave the intermediate compound (II), identical with that obtained on acid hydrolysis. Alkaline hydrolysis with 2% KOH gave the same product, but saponification took place faster.

Intermediate Product (II) (Orientin). mp 261-263°C; R_f 0.18 (system 1), 0.43 (system 2). UV spectrum (nm): λ_{max} (methanol), 259, 270, 351; + NaOMe:276, 408; + $AlCl_3$:278, 420; + $AlCl_3$ + HCl:264, 279, 300 d, 358, 385 sh.; + NaOAc:279, 393; + NaOAc + H_3BO_3:266, 374. IR spectrum (cm^{-1}): 3470-3380 (—OH); 1662 (C=O); 1618, 1520 (C=C); 1090, 1050, 1018 (pyranose form of

the sugar); PMR spectrum (DMSO, ppm, 25°C): 7.52 (m, H-6',2'); 6.90 (d, 8.5 Hz, H-5'); 6.68 (s, H-3); 6.31 (s, H-6); 4.73 (d, 10 Hz, H-1"); 3.60 (m, glucose protons).

Hydroxylaminolysis of (I). A mixture of 30 mg of substance (I) and 3-4 drops of a saturated solution of hydroxylamine hydrochloride in methanol was heated in a test-tube. After one drop of a saturated solution of KOH in methanol had been added the mixture was heated on the boiling water bath to the boil. Then it was cooled, acidified with 1% HCl, and chromatographed in system 1, the spots being revealed with a 1% ethanolic solution of ferric chloride. Acethydroxamic acid was detected in the form of a blue spot with R_f 0.90. The hydroxylaminolysis of ethyl acetate was carried out in parallel as a check. Similar results were obtained.

Detection of Acetic Acid. A solution of 200 mg of substanc (I) in 10 ml of 1% aqueous KOH was left at 20-22°C for 30 min. Then it was neutralized with 5% HCl to pH 5 and was extracted several times with diethyl ether. The aqueous solution yielded (I). The combined ethereal extracts were dried with anhydrous sodium sulfate and evaporated to small volume. The residue, smelling strongly of acetic acid (pH 1), was diluted with 3-4 drops of water, and diethylamine was added to pH 10. The diethylammonium acetate formed was identified by paper chromatography. As a marker, authentic diethylammonium acetate was chromatographed in parallel. The spots had a blue-green coloration and R_f 0.47 (system 3), 0.12 (system 4), 0.38 (system 5).

Acetylation of (I) and of Orientin. A mixture of 50 mg of (I) (or orientin), 0.5 ml of pyridine, and 0.5 ml of acetic anhydride was heated on the boiling water bath for 1 h. Then it was poured into 25 ml of ice-water, the precipitate that deposited was filtered off after 24 h, and it was washed with water to neutrality and recrystallized twice from 70% ethanol.

Acetate of (I). mp 200-202°C; a mixture with orientin acetate gave no depression of the melting point; R_f 0.21 (TLC, system 6). IR spectrum (cm^{-1}): 1765-1770 (—OAc); 1658 (C=O); 1612, 1505 (C=C). PMR spectrum ($CDCl_3$, ppm, 25°C): 7.55 (m, H-2',6'); 6.85 (d, 7 Hz, H-5'); 6.68 (s, H-3); 6.62 (s, H-6); 5.45 (m, H-1"); 4.12 (m, sugar protons); 2.45 (s, C_5—OAc); 2.38 (s, C_7—OAc); 2.37 (s, C_3',C_4'—OAc); 2.10 (s, 4"-OAc); 2.05 (s, 3"-OAc); 1.95 (s, 6"-OAc); 1.75 (d, 2"-OAc).

SUMMARY

From the epigeal part of hairy St. John's wort *Hypericum hirsutum* L. we have isolated a new acetylated C-glycoflavonoid for which the structure of luteolin 8-C-(2"-O-acetyl-β-D-glucopyranoside) (2"-O-acetylorientin) has been established.

LITERATURE CITED

1. G. Kitanov, Kh. Akhtardzhaev, and K. F. Blinova, Khim. Prir. Soedin., 269 (1978).
2. P. Lebreton and M. P. Bouchez, Phytochemistry, 6 (1967).
3. V. A. Bandyukova and A. L. Shinkarenko, The Thin-Layer Chromatography of Flavonoids [in Russian], Pyatigorak (1973), p. 8.
4. E. F. Bryant, J. Am. Pharm. Assoc., 39, No. 8, 481 (1950).
5. N. P. Maksyutina and V. I. Litvinenko, in: Phenolic Compounds and their Biological Functions [in Russian], Moscow (1968), p. 7.
6. T. J. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of the Flavonoids, Springer, New York (1970).
7. B. Gentili and G. Horowitz, J. Org. Chem., 33, No. 4, 1571 (1968).
8. W. E. Hillis and D. H. S. Horn, Austr. J. Chem., 18, No. 4, 531 (1965).
9. E. Bombardelli, A. Bonati, B. Galetta, and G. Mustich, Phytochemistry, 13, No. 1, 295 (1974).
10. M. I. Borisov, A. G. Serbin, and N. F. Komissarenko, Khim. Prir. Soedin., 281 (1972).
11. A. K. Jones, E. J. Dowling, and W. J. Scraba, Anal. Chem., 25, No. 3, 394 (1953).
12. V. I. Litvinenko, Rast. Res., 2, No. 4, 531 (1966).
13. L. P. Smirnova, G. G. Zapesochnaya, V. I. Sheichenko, and A. I. Ban'kovskii, Khim. Prir. Soedin., 313 (1974).
14. V. S. Batyuk, N. V. Chernobrovaya, and A. P. Prokopenko, Khim. Prir. Soedin., 90 (1966).
15. J. Fisel, Arzneim-Forsch., 15, 1417 (1965).
16. N. Nikolov, V. S. Batyuk, I. P. Kovalev, and V. Ivanov, Khim. Prir. Soedin., 116 (1973).
17. R. M. Horowitz and B. Gentili, Chem. Ind. (London), No. 9, 625 (1966).
18. A. B. Segelman, F. P. Segelman, A. E. Star, H. Wagner, and O. Seligmann, Phytochemistry, 17, No. 4, 824 (1978).

19. H. Wagner, L. Rosprim, and K. Galle, Phytochemistry, 14, No. 4, 1089 (1975).
20. R. A. Eade, W. E. Hillis, D. H. S. Horn, and I. J. H. Simes, Austr. J. Chem., 18, No. 5, 715 (1965).

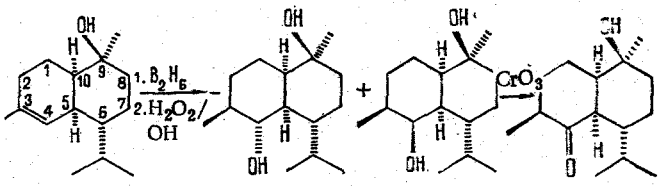
CRYSTAL AND MOLECULAR STRUCTURE OF THE SESQUITERPENE KETONE 9α-HYDROXYMUROLA-4-ONE

Yu. V. Gatilov, V. A. Khan, and Zh. V. Dubovenko

UDC 539.26:547.913.2

In a study of the structure and absolute configuration of bicyclic sesquiterpenoids of the cadalene series, some of their keto derivatives have been synthesized, and the optical activities of these derivatives have been studied by the ORD and CD methods [1]. However, the use of the octant rule for this set of ketones has led to ambiguous results, since their spatial structure has not been reliably established.

In the present paper we give results of an x-ray structural investigation of the ketone (IV) synthesized from (+)-δ-cadinol (I) and having mp 92-93°C.



Under the conditions of the oxidation of diols and, particularly, of the subsequent isolation of the ketone (IV) on sorbents, epimerization at two asymmetric carbon atoms C(3) and C(5) is possible on sorbents.

The structure of the molecule of the ketone (IV) is shown in Figure 1. The mean bond lengths and valence angles of two independent molecules having the same conformations are given below.

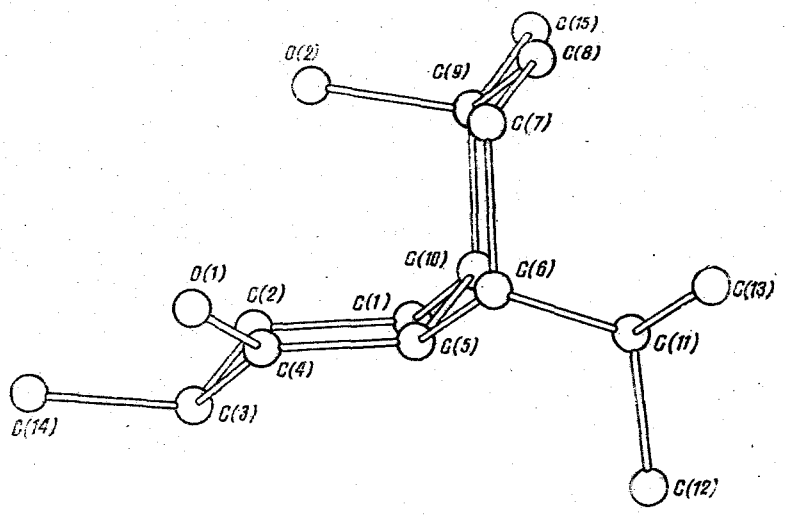


Fig. 1. Crystal structure of the sesquiterpenoid 9α-hydroxy-murola-4-one.

Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 158-161, March-April, 1979. Original article submitted November 24, 1978.

0009-3130/79/1502-0133$07.50