FLAVONOLIGNANS OF Salsola collina

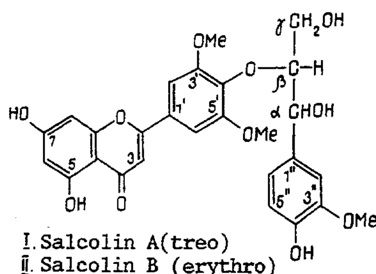
A. I. Syrchina, A. G. Gorshkov, V. V. Shcherbakov, S. V. Zinchenko, A. L. Vereshchagin, K. L. Zaikov, and A. A. Semenov

UDC 547.972

The new flavonolignans salcolin A [threo-4'-O-(β-guaiacylglyceryl)tricin] and salcolin B [erythro-4'-O-(β-guaiacylglyceryl)tricin] have been isolated from the epigeal part of Salsola collina Pall. (family Chenopodiaceae). The structures of the flavonolignans have been established on the basis of the results of NMR and mass spectrometry.

It has been established previously that in an aqueous alcoholic extract of Salsola collina Pall. (family Chenopodiaceae) tricin predominates among the flavonoid components. In the present paper we describe the isolation of two new tricin derivatives belonging to the flavonolignan group and the determination of their structures. They were present in an extract in minor amounts. The flavonolignans isolated were threo- and erythro- stereoisomers, and the names salcolin A (I) and salcolin B (II) have been proposed for them. Compounds (I) and (II) were isolated with the aid of column chromatography in the form of a mixture of the two and had identical R_f values, appearing as a single spot. The conclusion of the inhomogeneity of the samples made from an interpretation of the NMR spectra and was then confirmed by high-performance liquid chromatography. In the latter case it was possible under the conditions of reversed-phase chromatography to show the presence of two resolved peaks with R_s = 1.46 in a ratio of 1:1. Electronic absorption spectra obtained by the stopped-flow method were the same for the two components: λ_{max}, nm: 210, 270, 340.

A proof of the structures of (I) and (II) was made on mixture of them, and then, after separation by preparative HPLC, their spectral characteristics were obtained and the configurations of the individual components were suggested.



I. Salcolin A(treo)
II. Salcolin B (erythro)

The molecular mass of (I) and of (II), found with the aid of FAB mass spectrometry [m/z 527 $(M + H)^+$], in combination with spectral results, corresponded to the empirical formula $C_{27}H_{26}O_{11}$. Analysis of the ^1H and ^{13}C NMR spectra showed that (I) and (II) were composed of identical flavone and guaiacylglycerol fragments and differed only by the stereochemistry of the aliphatic part of the molecules. The flavone fragment was represented by a tricin residue. In the FAB-MS spectra of (I) and (II), in addition to the protonated molecular ions with m/z 527 $(M + H)^+$ there was, in each case, the peak of an ion with m/z 331 which is characteristic for tricin, while in the EI spectrum there were ions with m/z 330, 178, 153, 152 and m/z 196, 180, 138, 137, 124 belonging to fragments from the breakdown of the flavone and the guaiacylglycerol moieties of the molecule, respectively.

In the ^1H NMR spectrum taken in DMSO-d_6 the signals of eight aromatic protons were detected in the 6.23-7.32 ppm region, and also three one-proton singlets at 12.87 (5-OH), 10.88 (7-OH), and 8.76 (4"-OH). In total, the molecule of each of the components of the mixtures

---

Irkutsk Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences.
Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 182-186, March-April, 1992. Original article submitted July 4, 1991.

0009-3130/92/2802-0155$12.50 © 1992 Plenum Publishing Corporation

TABLE 1. ^{13}C NMR Spectrum of Each of the Flavonolignans (I) and (II) ($DMSO-d_6$, δ, ppm, TMS – 0)

Atom	δ, ppm	Atom	δ, ppm	Atom	δ, ppm
C-2	164,36	C-3′	153,04	C-1″	133,11* I 133,32* II
C-3	103,96	C-4′	139,64* I 139,99* II		
C-4	181,97			C-2″	111,11
C-5	161,49	C-5′	153,04	C-3″	147,08
C-6	99,03	C-6′	104,39	C-4″	145,46
C-7	163,17	C-α	71,73 I 72,21 II	C-5″	114,79
C-8	94,42			C-6″	119,45
C-9	157,48	C-β	87,06 I 86,57 II	$OCH_3 \times 2$	56,50
C-10	104,93			OCH_3	55,64
C-1′	125,41	C-γ	60,57 I 60,24 II		
C-2′	104,39				

*Assignment ambiguous.

TABLE 2. ^1H NMR Spectra of the Flavonolignans (I) and (II) (CD_3CN, TMS – 0, δ, ppm, J, Hz)

Protons	I+II (200 MPa)	threo-(I) (500 MHz)	erythro- (II) (500 MHz)
H-2′, 6′	7,30 (2H, s)	7,32 (2H, s)	7,32 (2H, s)
H-2″	7,04 (1H, br.s)	7,05 (1H, d, 1,7)	7,04 (1H, d, 1,7)
H-6″	6,79–6,90 (3H, m)	6,89 (1H, dd 1,7 & 8)	6,91 (1H, dd 1,7 & 8)
H-5″		6,84 (1H, d 8)	6,82 (1H, d, 8)
H-3		6,80 (1H, s)	6,81 (1H, s)
H-8	6,62 (1H, d, 2)	6,63 (1H, d, 2,2)	6,63 (1H, d, 2,2)
H-6	6,30 (1H, d, 2)	6,31 (1H, d, 2,2)	6,31 (1H, d, 2,2)
3′, 5′-OCH_3	3,98 (3H, s) 4,00 (3H, s)	4,00 (6H, s)	4,07 (6H, s)
3″-OCH_3	3,88 (1,5H, s) 3,90 (1,5H, s)	3,90 (3H, s)	4,02 (3H, s)
5-OH	12,88 (1H, s)	12,8 (1H, s)	12,8 (1H, s)
H-α	5,0 (1H, br.d)	5,0 (1H, d, 4,9)	5,0 (1H, d, 7)
H-β	4,37 (0,5H, m) 4,20 (0,5H, m)	4,36 (1H, m)	4,23 (1H, m)
H-$γ_{1,2}$	3,34–3,80 (2H, m)	3,55 (1H, dd) 3,3 & 12,3) 3,85 (1H, dd) 5,3 & 12,3)	3,70 (1H, dd), 3,5 & 22,4) 3,38 (1H, dd 3,0 & 12,4)

of (I) and (II) contained five hydroxy groups, since acetylation led to mixtures of the pentaacetates of (I) and (II), each with a molecular mass M^+ of 736, were obtained. Two hydroxy groups were attached to aliphatic C-atoms as was shown by the presence of signals at 2.0 ppm (6H, s) in the ^1H NMR spectra of the pentaacetates.

In the ^{13}C NMR spectrum all the signals of the carbon atoms of the flavone and guaiacyl moieties of (I) and (II) were observed (Table 1). Only the carbon atoms of the aliphatic part of the guaiacylglycerol moiety resonated in the form of doubled signals, as also did the C-4' and C-1" carbons.

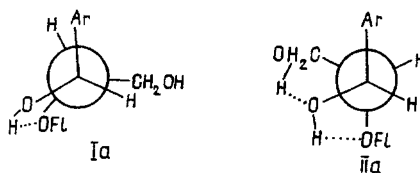
The position of attachment of the flavone fragment to the β-OH of the guaiacyl was determined from a comparison of the chemical shifts of the signals of the carbons of the aliphatic moiety with literature information for guaiacylglycerol derivatives: the flavonolignan aegicin [2] and the cerberalignans F and H [3] and G, J, M, and N [3, 4], having the erythro- and threo-configurations, respectively. The assignment of the CSs of the signals of the carbon atoms of the flavone and phenyl moieties of the flavonolignans (I) and (II) was made on the basis of a comparison of the CSs of tricin, tricetin, and 3',4',5'-trimethoxytricetin [5] and guaiacylglycerol derivatives [2-4].

Information on the stereochemistry was obtained from the ^1H NMR spectrum of the mixture of (I) and (II) taken in CD_3CN (Table 2). In the spectrum, two groups of signals of the H-β and H-γ protons were distinguished for (I) and (II), while the H-α signal appeared in the form of a broadened doublet.

To separate the mixture of isomers (I) and (II) we used preparative HPLC. As a result, the individual components (I) and (II) were isolated in amounts of ~1.5 and 1.6 mg, respectively. The ^1H NMR spectra (see Table 2) and also the EI mass spectrum of the isolated isomers were recorded.

The limited solubility of the individual substances in acetonitrile must be mentioned. However, only in this solvent was it possible to obtain spectra with a high resolution of all the signals of the aliphatic moiety with first-order spin-spin coupling, in contrast to the spectra taken in DMSO or pyridine, where the resolution of the signals was considerably worse and some of them were masked by the signals of the solvent.

The conclusion concerning the configurations of the individual substances was made on the basis of SSCCs (see Table 2), starting from the assumption that the almost eclipsed conformation (Ia) is the most stable for the threo- isomer and the skew conformation (IIa) for the erythro- isomer. In these conformations, the voluminous substituents are as far from each other as possible and additional stabilization takes place through hydrogen bonds. The observed SSCCs correspond to the dihedral angles existing in these conformations.



Thus, on the basis of what has been said above it has been established that salcolin A (I) and salcolin B (II) are new stereoisomeric flavonolignones having the structure of 4'-O-(β-guaiacylglyceryl)tricin, for which the threo- and erythro- configurations, respectively, have been proposed.

At the present time, three similar compounds are known in which the flavonoid and arylglycerol fragments are linked by an oxygen bridge between the C-4' and C-β atoms: aegicin [2] and lignoside and isolignoside [6].

EXPERIMENTAL

NMR spectra were recorded on the following spectrometers: JEOL FX-90Q (^1H, 89.55 MHz; ^{13}C, 22.49 MHz) in DMSO-d_6 (δ, 0 – TMS); Bruker WP-200 (200.13 MHz) in CD_3CN; and Varian WXR-500s (499.84 MHz). FAB–MS spectra were obtained on an LKB-2091 instrument with FAB by an Iontech ion source. Ionization by Xe atoms with an energy of 6 keV with a discharge current of 1 mA; EI spectra were taken at an ionization energy of 70 eV and a direct-inlet temperature of 300°C. HPLC analysis was performed on a Milikhrom-1 chromatograph with a 2 × 64 mm column containing the stationary phase Separon-5, C-18, with methanol–water (60:40) as the mobile phase.

Isolation of a Mixture of Flavonolignans (I) and (II). Substances (I) and (II) were obtained simultaneously with the isolation of tricin by the chromatography on polyamide of an ethyl acetate extract of the butanol fraction [1], being eluted from the column with chloroform–methanol (85:15, vol. %). They were purified on columns of silica gel with the use of chloroform–methanol (0-5%) and also benzene–chloroform (1:1) with the addition of methanol (0-20%) to it. The ratio of substance to sorbent ranged from 1:200 to 1:300. From 80 kg of raw material we isolated ~90 mg of the mixture of (I) and (II).

Separation of the Mixture of (I) and (II) by the HPLC Method. Preparative separation was achieved on a Bruker LC chromatograph with a 25 × 250 mm column containing Nucleosil-5, C-18 in the isocratic regime using acetonitrile–water (50:50) as the mobile phase. Detection was carried out at 320 nm. The mixture to be separated was dissolved in 100% methanol. The volume of the sample introduced was 200 μl with a concentration of 10 mg/ml. The amounts of flavonolignans (I) and (II) isolated were 1.5 and 1.6 mg.

Threo-4'-O-(β-Guaiacylglyceryl)tricin (Salcolin A) (I). $C_{27}H_{26}O_{11}$, yellow powder, EI-MS, m/z, %: 508 [$(M - H_2O)^+$, 0.16]; 490 [$(M - 2 \times H_2O)$, 0.12]; 330 ($M - C_{10}H_{12}O_4$, 100); 301(3.36); 302(2.21); 287(5.33); 259(5.0); 244(3.0); 243(1.0); 229(1.5); 216(3.5); 213(4.2); 196(2.1); 187(1.9); 181(2.5); 180(15.2); 179(2.9); 178(11.0); 163(5.2); 161(1.4); 153(12.8); 152(3.6); 151(16.3); 138(2.9); 137(22.8); 135(5.9); 124(16.6); 123(6.4); 120(4.1); 119(6.7);

118(1.7); 117(1.9); 109(4.3); 108(3.7); 105(3.4); 103(4.8); 91(10.2); 89(4.5); 79(5.2); 77(8.7); 69(10.3); 65(6.5); 63(7.3); 55(9.9); 43(7.3); UV, λ_{max}, nm: 270, 340; A_{260}/A_{350} = 0.836. ^1H NMR (DMSO-d_6), δ, ppm: 6.20-7.35 (8H, aromatic protons), 4.78 (1H, d, J = 5 Hz H-α), 4.32 (1H, m, H-β), 3.74 (3H, s, 3"-OCH_3), 3.86 (6H, s, 3',5'-OCH_3), 12.84 (1H, s, 5-OH); for the ^1H NMR spectrum in CD_3CN, see Table 2.

Erythro-4'-O-(β-Guaiacylglyceryl)tricin (Salcolin B) (II). $C_{27}H_{26}O_{11}$, yellow powder, EI-MS, m/z, %: 508 [$(M - H_2O)^+$, 17.5]; 490 [$(M - 2 \times H_2O)^+$, 14.6]; 331(17.2); 330(100); 329(38.2); 302(1.9); 301(4.8); 287(5.8); 259(7.8); 244(3.9); 241(3.9); 229(3.6); 216(4.2); 215(3.6); 213(6.2); 196(1.0); 187(3.9); 181(2.3); 180(8.8); 179(5.2); 178(14.2); 163(9.7); 161(2.3); 153(17.5); 152(2.9); 151(13.3); 138(3.2); 137(20.5); 135(10.7); 124(10.7); 123(7.1); 122(4.2); 120(6.2); 119(4.2); 118(4.9); 117(4.9); 109(4.2); 108(4.5); 107(5.8); 106(4.9); 105(4.9); 103(4.5); 91(7.8); 89(7.1); 79(7.9); 78(6.5); 77(12.7); 69(18.8); 65(7.8); 63(10.0); 55(18.5); 53(10.0); 43(23.7); UV, λ_{max}, nm: 270, 340; A_{260}/A_{350} = 0.847; ^1H NMR (DMSO-d_6), δ, ppm: 6.20-7.35 (8H, aromatic protons), 4.83 (1H, d, J = 6.5 Hz, H-α), 4.20 (1H, m, H-β), 3.84 (6H, s, 3',5'-OCH_3), 3.72 (3H, s, 3"-OCH_3), 12.8 (1H, s, 5-OH); for the ^1H NMR spectrum in CD_3CN, see Table 2.

Mixture of (I) and (II). $C_{27}H_{26}O_{11}$, yellow powder with no clear melting point. FAB-MS, m/z 527 $(M + H)^+$, 331 $(M + H - C_{10}H_{12}O_4)^+$; EI-MS, m/z (%), 330(25.1); UV, λ_{max} (CH_3OH—H_2O, 6:4): 210, 270, 340 nm; A_{260}/A_{350} = 0.836 (I), A_{260}/A_{350} = 0.847 (II). ^1H NMR (DMSO-d_6), δ, ppm: 3.74 and 3.76 (each 1.5H, s, 3"-OCH_3), 3.87 and 3.89 (each 3H, s, 3',5'-OCH_3), 4.3 (1H, m, H-β), 4.9 (1H, m, H-α), 6.23 (1H, d, J = 2 Hz, H-6), 6.58 (1H, d, J = 2 Hz, H-8), 6.70-7.34 (6H, aromatic protons), 8.76 (1H, s, 4"=OH), 10.88 (1H, s, 7-OH), 12.87 (1H, s, 5-OH); for the ^{13}C NMR spectrum, see Table 1.

Acetylation of (I) and (II). The mixture of (I) and (II) (40 mg) was acetylated with acetic anhydride in the presence of pyridine (20°C, 30 h) and, after the addition of ice to the reaction mixture, the precipitate was filtered off and was recrystallized from ethanol. The resulting mixture was chromatographed on columns of silica gel in the benzene—acetone (0-50%) system. Mixtures of the pentaacetates of (I) and (II) (7 mg) and mixtures of the tetra- and pentaacetates of (I) and (II) (~20 mg) were isolated. The course of separation was monitored by the TLC and FAB—MS methods.

Pentaacetates of (I) and (II). $C_{37}H_{36}O_{16}$, colorless crystals: ^1H NMR (89.55 MHz), $CDCl_3$, δ, ppm: 2.0 (6H, s, aliphatic OAc), 2.15 (3H, s, 4"-OAc), 2.31 (3H, s, 7-OAc), 2.41 (3H, s, 5-OAc), 3.83 (9H, br.s, 3 × OCH_3), 3.9-4.33 (2H, m, H-$\gamma_{1,2}$), 4.70 (1H, m, H-β), 6.13 (1H, m, H-α), 6.52-7.26 (8H, aromatic protons).

LITERATURE CITED

1. A. I. Syrchina, A. L. Vereshchagin, M. F. Lapin, and A. A. Semenov, Khim. Prir. Soedin., No. 5, 527 (1989).
2. R. Cooper, H. E. Gottlieb, and D. Lavie, Israel J. Chem., 16, 12 (1977).
3. F. Abe, Y. Yamauchi, and A. S. C. Wan, Phytochem., 27, No. 11, 3627 (1988).
4. F. Abe, Y. Yamauchi, and A. S. C. Wan, Phytochem., 28, No. 12, 3473 (1989).
5. K. R. Markham, B. Ternai, R. Staney, et al., Tetrahedron, 34, 1389 (1978).
6. V. A. Kurkin and G. G. Zapesochnaya, Khim. Prir. Soedin., No. 1, 11 (1987).