STRUCTURE OF CAULOSIDE B – A GLYCOSIDE OF A NEW TRITERPENOID CAULOPHYLLOGENIN FROM *Caulophyllum robustum*

L. I. Strigina, N. S. Chetyrina,
V. V. Isakov, A. K. Dzizenko,
and G. B. Elyakov

UDC 547.918+547.597

We have previously reported the isolation from Caulophyllum robustum Maxim. of four glycosides of hederagenin (caulosides A, C, D, and E) and of cauloside B – the glycoside of an unidentified triterpenoid which we have called caulophyllogenin [1].

The IR spectrum of caulophyllogenin obtained by the hydrolysis of cauloside B with 2 N H_2SO_4 in ethanol contained the bands of a hydroxy group (3595 cm^{-1}) and of a carboxy group (1705 cm^{-1}). The presence of the latter was confirmed by the preparation of the corresponding methyl ester (Ib). We obtained preliminary information on the structure of caulophyllogenin by a consideration of its mass spectrum. In the mass spectrum of (Ia) (Table 1), in addition to the peak of the molecular ion (M^+ 488) intense peaks of ions characteristic for the retro-Diels–Alder type of fragmentation of triterpenoids of the olean-12-ene type with a carboxy and one of the hydroxy groups in rings D/E [2] were observed. The treatment of (Ia) with acetone and p-toluenesulfonic acid gave a monoacetonide (Ii), the mass spectrum of which (see Table 1) showed the presence in it of an acetonide group and, consequently, the presence of the corresponding two hydroxy groups in rings A/B. The acetylation of (Ib) under the usual conditions gave (according to the results of thin-layer chromatography) a mixture of two acetates (c and d). On reacetylation of the mixture with heating, the amorphous acetate (Ic) was formed, with an IR spectrum in which the absorption of a hydroxy group was absent. The NMR spectrum of (Ic) (Table 2) showed that the substance contained six tertiary methyls, one methoxycarbonyl, and three acetyl groups. In the weak-field region the signals of one proton on a double bond and signals showing the presence of one acetoxymethyl and two acetoxymethylene groups appeared. The acetylation of (Ib) at 0°C gave an amorphous acetate (Id), in the IR spectrum of which the absorption band of a hydroxy group appeared (3630 cm^{-1}). The NMR spectrum of (Id) (see Table 2) showed that the substance contains two acetyl groups; one of them forms part of an acetoxymethyl and the other of an acetoxymethylene group. In the weak-field region was observed the signal of a proton attached to the same carbon atom as the hydroxy group.

Consequently, in caulophyllogenin there are one primary and two secondary hydroxy groups, one of the latter not being acetylated under the usual conditions.

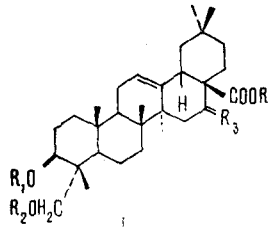
The hydrolysis of cauloside B with a mixture of concentrated HCl and MeOH (1:1) formed another aglycone which, according to its IR spectrum (1755 cm^{-1}) was the γ-lactone (II). The presence in the mass spectrum of (IIa) of the peak M^+ 488, and increase in the intensity of the peaks with m/e 236 and 223, and the absence of the absorption band of a double bond in the IR spectrum of the γ-lactone showed that the lactone group in it arose as the result of ring closure by the carboxy group with the Δ^{12} double bond during acid hydrolysis. In the acetylation of the lactone under ordinary conditions a mixture of acetates was obtained from which an amorphous triacetate (IIb) was isolated [NMR: δ 2.1, 2.15, 2.5 ($-3CO-CH_3$)]. On the basis of these facts it was assumed that caulophyllogen is structurally similar to hederagenin but bears an additional

TABLE 1. Mass Spectra of (Ia), (Ie), and (Ii)

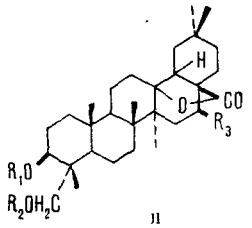
Ia		Ie		Ii	
m/e	Relative intensity, %	m/e	Relative intensity, %	m/e	Relative intensity, %
488	8	584	25	528	7
264	56	276	100	264	73
246	100	—	—	246	100
218	32	216	44	—	—
201	50	199	88	201	40
200	16	—	—	200	86

Pacific Ocean Institute of Bioorganic Chemistry of the Far-Eastern Scientific Center, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 733-738, November-December, 1974. Original article submitted July 12, 1973.

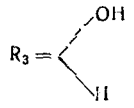
hydroxy group in ring D/E which experiences hindrance on acetylation. In actual fact, the oxidation of the diacetate of the methyl ester (Id) with chromium trioxide in pyridine gave a ketone (Ie). The latter, according to its IR, mass, and NMR spectra (see Tables 1 and 2) contains a carbonyl group in rings D/E. It has been found previously that the CD curve of ketone (Ie) shows a strong negative Cotton effect ($\Delta\varepsilon_{305} - 3.7$, $\Delta\varepsilon_{296} - 3.68$), comparable with that of methyl 16-oxo-24-norolean-12-en-28-oate [6]. The 16α configuration of the hydroxy group was shown by a direct correlation of (Ia) and (Ib) with the product of the reduction by $NaBH_4$ of quillaic acid* and the corresponding methyl ester.

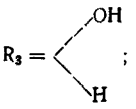


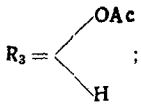
I

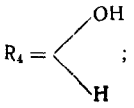


II

Ia. $R_1 = R_2 = R = H$; $R_3 =$ 

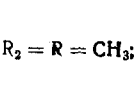
b. $R_1 = R_2 = H$; $R_3 =$  ; $R = CH_3$

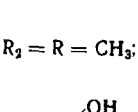
c. $R_1 = R_2 = Ac$; $R_3 =$  ; $R = CH_3$

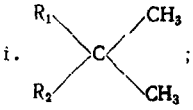
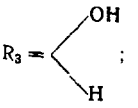
d. $R_1 = R_2 = Ac$; $R_4 =$  ; $R = CH_3$

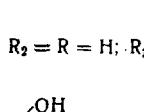
e. $R_1 = R_2 = Ac$; $R_3 = O$; $R = CH_3$

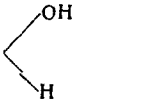
f. $R_1 = R_2 = H$; $R_2 = H_2$; $R = CH_3$

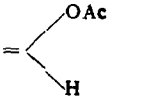
g. $R_1 = H$; $R_2 = R = CH_3$; $R_3 =$ 

h. $R_1 = Ac$; $R_2 = R = CH_3$; $R_3 =$ 

i.  ; $R_3 =$  ; $R = H$

j. $R_1 = -\alpha - L - Ar$; $R_2 = R = H$; $R_3 =$ 

IIa $R_1 = R_2 = H$; $R_3 =$ 

b. $R_1 = R_2 = Ac$; $R_3 =$ 

To establish the structure of cauloside B we obtained its permethylate by Hakomori's method [7]. The acid hydrolysis of the latter gave 2,3,4-tri-O-methyl-L-arabinose and an amorphous aglycone (Ig). The NMR spectrum of the acetate of (Ig) (Ih) showed the signals of the protons of two methoxy, one methoxycarbonyl, and one acetyl group (see Table 2). The latter is present at the C_3 atom (signal of a proton in the weak-field region at 4.85 ppm, $J_{AX} + J_{BX} = 14.5$ Hz), which shows the position of attachment of the arabinose.

*A sample of quillaic acid was kindly given to us by Prof. N. K. Tsawa (Shionogi Company, Osaka, Japan).

TABLE 2. Chemical Shifts (δ) of the Signals of the Protons in the NMR Spectra of the Transformation Products of Caulophyllogenin (Ia)

Compound	Methyl group	-C-CH_3	-O-CO-CH_3	-COO-CH_3	CH_2-OAc	3α-H	16-β-H	12-H	-18-H	OCH_3
Ic	26	0,65(3H)	1,88(3H)	3,54(3H)	3,66(2H br. s.)	4,61(T)	5,47(1H)	5,32(1H br. s.)	2,92	
	23	0,77(3H)	1,96(6H)							
	25	0,89(3H)								
	29/30	0,93(6H)								
	27	1,15(3H)								
Id	26	0,66(3H)	1,91(3H)	3,52(3H)	3,60(2H br. s.)	4,65(T)	4,46(1H)	5,29(1Hbr. s.)	2,93	
	23	0,78(3H)	1,96(3H)							
	25	0,86(3H)								
	29/30	0,94(6H)								
	27	1,32(3H)								
Ie	26	0,83(9H)	2,03(3H)	3,69(3H)	3,81(2H br. s.)	4,82(T)		5,56(1Hbr. s.)		
	23/25		2,06(3H)							
	29	0,91(3H)								
	30	0,98(3H)								
	27	1,17(3H)								
Ih	26	0,66(3H)	1,93(3H)	3,54(3H)	3,2—3,4	4,85(T)	3,87(1H)	5,2(1H br. s.)		3,22(3H)
	23	0,68(3H)								3,31(3H)
	25	0,87(3H)								
	29/30	0,94(6H)								
	27	1,24(3H)								

The presence of the signal of the anomeric proton at δ 4.40 ppm, J 5.5 Hz, in the NMR spectrum of the full acetate of cauloside B shows the α-configuration of the glycosidic bond.

EXPERIMENTAL

The chromatographic purification of the substances and the analysis of the reaction products were performed on KSK silica gel in the following solvent systems: 1) benzene—ethyl acetate (100 : 1 → 1 : 2); 2) chloroform—methanol (7 : 1); 3) toluene—ethanol (9 : 1); and 4) chloroform—ethanol (5 : 1). The acid hydrolysis of cauloside B and of the product of its methylation were performed by heating the substances for 2-5 h with the following mixtures: 5) 2 N H_2SO_4 in ethanol; 6) concentrated HCl in methanol (1 : 1); and 7) 42% $HClO_4$—methanol (1 : 5). Acetylation was performed with a mixture of acetic anhydride and pyridine (1 : 2). The IR spectra were recorded on a UR-10 spectrophotometer, the mass spectra on a MKh-1303 instrument, and the NMR spectra on a ZKR-60 instrument in $CHCl_3$ at 60 MHz (the chemical shifts are expressed in ppm on the δ scale). Abbreviations used: s — singlet, t — triplet.

Aglycone (Ia). Cauloside B (0.5 g) was hydrolyzed with mixture 5. The reaction mixture was diluted with water, the ethanol was distilled off, and the precipitate of the aglycone (Ia) was filtered off, washed free from acid, and dried; mp 277-282°C, $[\alpha]_D^{20}$ +14.3° (pyridine); IR spectrum, cm^{-1}: 1680 (C=C), 1705 (COOH), 3630 (OH).

Methyl Ester of the Aglycone (Ia) (Ib). Compound (Ia) (250 mg) was methylated with CH_2N_2 in methanol. This gave (Ib) with the composition $C_{31}H_{50}O_5$, mp 235-236°C (benzene—hexane), $[\alpha]_D^{20}$ + 33.32° (c 0.18; benzene). IR spectrum, cm^{-1}: 1730 ($COOCH_3$), 3630 (OH).

Aglycone (IIa). The aglycone (IIa) was formed under conditions 7. Mass spectrum: M^+ 488. Strong peaks with m/e 470 (M^+-H_2O), 452 (M^+-2H_2O), 439 ($M-H_2O-CH_2OH$), 236, 223, 218. On acetylation under the usual conditions, the aglycone (IIa) gave (IIb) (syrup).

Acetonide of (Ia) (Ii). A mixture of 136 mg of (Ia), 10 mg of p-toluenesulfonic acid, and 10 ml of absolute acetone was boiled for 48 h. By chromatography on silica gel in system 1 (10 : 1 → 7 : 1) compound (Ii) was isolated, with mp 136-138°C (ethyl acetate); $[\alpha]_D^{20}$ +19.5° (c 0.17; chloroform); IR spectrum, cm^{-1}: 1705 (COOH), 3630 (OH).

Acetylation of (Ib). A) Compound (Ib) (50 mg) was acetylated under the conditions given above by chromatography on silica gel in system 1 (100 : 0 → 80 : 1); 10 mg of (Ic) and 40 mg of (Id)

(syrups) were isolated. IR spectrum of (Ic), (cm^{-1}): 1745 ($COOCH_3$), ($-OCOCH_3$) (OH absent). B) Compound (Ib) (160 mg) was acetylated at 0°C for 24 h. This gave (Id) (syrup). IR spectrum, cm^{-1}: 1745 ($-O-COCH_3$), 3630 (OH).

Oxidation of (Id). A solution of 100 mg of (Id) in 2.5 ml of pyridine was treated with 100 mg of CrO_3 in 2.5 ml of pyridine and the mixture was stirred for 24 h. Chromatography was performed on silical gel in system 1 (100 : 0 → 50 : 1). This gave (Ie), $C_{35}H_{53}O_7$, mp 174-176°C (methanol), $[\alpha]_D^{20} - 5.94 + 2°$ (chloroform), IR spectrum, cm^{-1}: 1725 (C=O), 1750 ($-O-COCH_3$) cm^{-1}.

Reduction of (Ie). At 180°C, 235 mg of Na was dissolved in 11.06 ml of diethyleneglycol, and then 8.25 ml of hydrazine was added and the mixture was heated to 180°C with the distillation off of the hydrazine. To this reaction mixture was added 0.06 g of (Ie) in diethyleneglycol, and heating at 180°C was continued for 12 h. Then the temperature of the bath was raised to 210°C, a small amount of hydrazine was distilled off, and heating was continued for 24 h. After this, the reaction mixture was cooled, diluted with water, neutralized with 3% HCl, and extracted with ether. Chromatography on silica gel in system 1 (10 : 1 → 5 : 1) and methylation with CH_2N_2 gave (Ih) (identical in melting point and IR spectrum with the methyl ester of hederagenin).

Reduction of Quillaic Acid. A solution of 10 mg of quillaic acid in 1 ml of methanol was treated with 25 mg of $NaBH_4$ and the mixture was heated for 10 h. Then it was diluted with water, acidified with 3 N HCl, and extracted with ether. This gave a substance with mp 276-282°C. A mixture with the aglycone (Ia) gave no depression of the melting point. The substance was methylated with CH_2N_2. From its melting point and IR spectrum, the methyl ester was identical with (Ib).

Methylation of Cauloside B. Sodium methylsulfinylmethylide [8] was added dropwise to a solution of 320 mg of cauloside B in 10 ml of dimethyl sulfoxide. The gel formed was stirred for 2 h and then, with cooling, 5 ml of CH_3I was added, and stirring was continued for another 12 h. The methylation products were analyzed in system 3. After purification on silica gel, 145 ml of permethylate was obtained with $[\alpha]_D^{20} + 6.9 \pm 2°$ (c 1.9; benzene); IR spectrum: OH absent. 2,3,4-Tri-O-methyl-L-arabinose was identified (TLC, GLC). The thin-layer chromatography of the 2,3,4-tri-O-methyl-L-arabinose was performed in systems 2 and 4 on Silufol brand plates (150 × 150).

SUMMARY

It has been shown that caulophyllogenin is 3β,16α,23-trihydroxyolean-12-en-28-oic acid, and cauloside B is the 3-O-α-L-arabopyranoside of caulophyllogenin.

LITERATURE CITED

1. L. I. Strigina, N. S. Chetyrina, and G. B. Elyakov, Khim. Prirodn. Soedin., 552 (1970).
2. T. Kubota and H. Kitatani, Chem. Comm., 1005 (1968).
3. D. H. R. Barton and D. Ives, J. Chem. Soc., 2056 (1955).
4. C. Djerassi, C. H. Robinson, and D. B. Thomas, J. Amer. Chem. Soc., 78, 5685 (1956).
5. D. H. R. Barton and C. J. W. Brooks, J. Chem. Soc., 257 (1951).
6. J. Rondest and J. Polonsky, Bull. Soc. Chim. Fr., 1253 (1963).
7. S. Hakomori, J. Biochem., 55, 2 (1965).
8. E. J. Corey and M. Chaykovsky, J. Amer. Chem. Soc., 87, 1345 (1965).