LITHOSPERMOSIDE AND DASYCARPONIN, CYANOGLUCOSIDES FROM *THALICTRUM*

JINN WU, EDWARD H. FAIRCHILD, JACK L. BEAL, TOSHIAKI TOMIMATSU and RAYMOND W. DOSKOTCH

Division of Pharmacognosy and Natural Products Chemistry, College of Pharmacy, Ohio State University, Columbus, Ohio 43210

ABSTRACT.—Two cyanoglucosides from *Thalictrum* are assigned structures by physical and chemical methods. The glycoside from *T. rugosum* and *T. revolutum* is identical with lithospermoside and griffonin. Its absolute stereochemistry was determined as in **1** from circular dichroism studies with the dibenzoate of the modified aglycone, griffonilide (**2**). The other glycoside, dasycarponin (**5**), from *T. dasycarpum* is the 4-epimer of lithospermoside. Proton and carbon nuclear magnetic resonances, circular dichroism and other spectral data are given for these compounds and their products.

In 1965, while investigating the alkaloids of *Thalictrum rugosum* Ait. (*T. glaucum* Desf.) and *T. dasycarpum* Fisch. and Lall. (Ranunculaceae), two cyanoglycosides were isolated from the alcohol extracts of the roots and satisfactory elemental analyses obtained. A study by D. H. Lee in another laboratory established the formula by mass spectrometry for both as $C_{14}H_{19}NO_8$, and the sugar as *D*-glucose anomerically β-linked (1). The structures proposed for the aglycones, however, were tentative and incorrect. More recently, the glycoside originally obtained from *T. rugosum* was found in *T. revolutum* DC, and we undertook a reinvestigation of the structures of these compounds.

The glycoside from *T. rugosum* and *T. revolutum* was shown to be identical with lithospermoside reported from *Lithospermum purpureo-caeruleum* and *L. officinale* (Boraginaceae) (2) and with griffonin[1] from *Griffonia simplicifolia* Baill. (Caesalpinaceae) (3). Identity was established by direct comparison with authentic samples. The accepted structure, excluding absolute stereochemistry, is as shown in **1**. Assignment of structure by both groups of investigators was made from extensive spectral analyses, in particular, high resolution nuclear magnetic resonance (nmr). In addition, the modified aglycone, griffonilide (**2**), obtained by Dwuma-Badu *et al.* (3) from griffonin, was analyzed by X-ray crystallography, but only relative stereochemistry was determined (4). The six-membered ring, in griffonilide, exists in a stable half-chair conformation placing the hydroxyl groups equatorial at carbons 4 and 5.

By utilization of the "dibenzoate chirality rule" (5), a specific example of the "exciton chirality method" (6), we could assign the absolute chirality to the vicinal glycol unit. Griffonilide dibenzoate (**3**) exhibited a circular dichroism (cd) curve (figure 1) with two Cotton effects, a negative maximum at 238 nm, $[\theta]$ $-132{,}000$ ($\Delta\epsilon$ -40.0) and a positive maximum at 222 nm, $[\theta]$ $+56{,}400$ ($\Delta\epsilon$ $+17.1$). This pattern of Davydov splitting of the two $\pi\rightarrow\pi^*$ benzoyl chromophores, in which the higher wavelength Cotton effect maximum is negative, corresponds to a negative or counterclockwise chirality. Thus, the arrangement of the dibenzoate groups must be as drawn in **4**, requiring a 4*R*, 5*S*, 6*S* absolute configuration for

[1]We have chosen the name lithospermoside over griffonin for this compound on the basis of priority established by the date of manuscript receipt over the date of publication. This is the first appearance of a name for the substance in a scientific journal and supercedes the name thalrusine used by Lee (1).

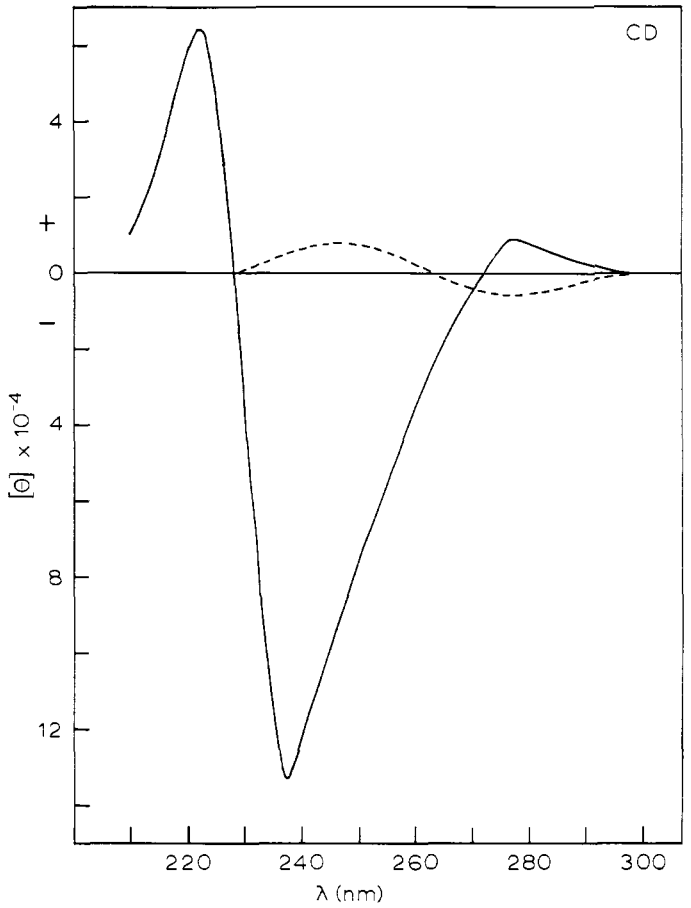


FIG. 1. Circular dichroism curves for griffonilide (**2**) (- - -) and griffonilide dibenzoate (**3**) (———).

griffonilide (**2**). Lithospermoside, therefore, has absolute stereochemistry as drawn in **1**.

The second glycoside, dasycarponin[2] (**5**) mp 253–5°(d), $[\alpha]D$ −11°, crystallized from the crude ethanolic extract of *T. dasycarpum* roots. Its composition, $C_{14}H_{19}NO_8$, as established by elemental analyses and mass spectrometry, is the same as that of lithospermoside (**1**); and similar spectral properties suggested a close structural relationship. The infrared spectrum shows a peak at 2230 cm^{-1} for the cyano group; and the proton (^1H) nmr spectrum (see Experimental) contains the further split AB quartet at δ 6.35 and 6.05 for H–2 and H–3, respectively, and the H–7 proton at δ 5.70. The carbon (^{13}C) nmr spectrum (table 1) is little different from that of lithospermoside. Acetylation of dasycarponin gave a hexaacetate differing little in properties from the acetate of lithospermoside.

Hydrolysis of dasycarponin (**5**) by the almond β-glucosidase, emulsin, or by acid yielded the modified aglycone dasycarponilide (**6**) and *D*-glucose, establishing

[2]This name has been chosen over that of thaldasine used by Lee (1) to avoid confusion with the alkaloids thalidasine and thalidezine.

TABLE 1. Carbon nuclear magnetic resonance peaks for lithospermoside (**1**) and dasycarponin (**5**).[a]

Carbon	Lithosperm-oside	Dasycarponin	Carbon	Lithosperm-oside	Dasycarponin	Methyl β-*D*-glucopyran-oside[b]
1	157.6 s	155.4 s	1'	104.9	105.3	104.6
2	129.2	128.5	2'	75.3	75.6	74.6
3	138.7	139.8	3'	78.4	78.6	77.4
4	76.2	68.1	4'	72.3	71.3	71.2
5	78.5	79.4	5'	78.3	78.3	77.3
6	72.3	72.0	6'	63.5 t	63.2 t	62.4
7	99.4	102.5				
8	120.1 s	120.1 s				

[a]Spectra were taken in D_2O at ambient temperature (20–25°). Chemical shifts are in ppm with tetramethylsilane as internal standard as determined by broad band decoupling. Peak multiplicity was determined by off-resonance decoupling; all peaks are doublets except where indicated by s = singlet and t = triplet.

[b]Values measured in H_2O at 50 ± 5° and taken from reference 7.

the glycoside as a β-*D*-glucoside. Confirmation of the anomeric carbon stereochemistry was obtained from the chemical shift for carbon C-1' at δ_c 105.3 ppm; methyl glycosides of α- and β-*D*-glucopyranose have reported values of δ_c 100.6 and 104.6 ppm, respectively (7). The cd curve is the one spectral characteristic that is different for the two glycosides. Dasycarponin (**5**) showed a positive maximum at 277 nm and a negative maximum at 230 nm (fig. 2), a curve opposite

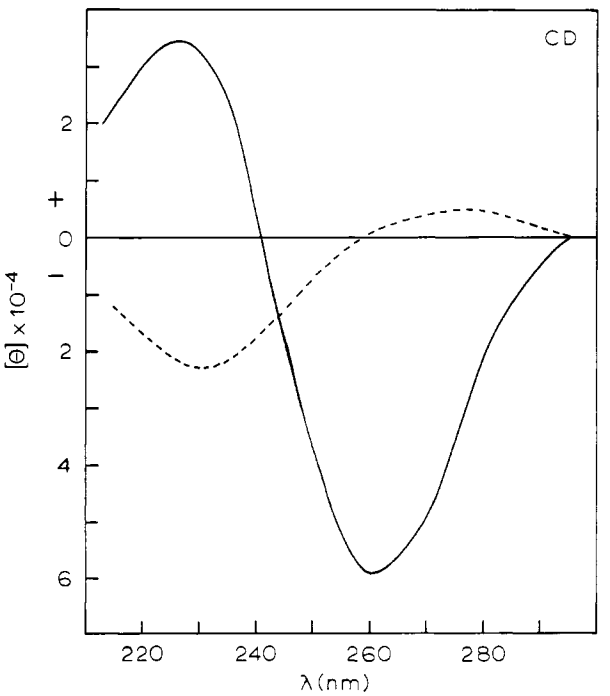


FIG. 2. Circular dichroism curves for lithospermoside (**1**) (———) and dasycarponin (**5**) (– – –).

TABLE 2. Proton magentic resonance peaks for griffonilide (**2**), dasycarponilide (**6**) and their derivatives.[a]

Compound	H-2	H-3	H-4	H-5	H-6	H-7	Miscellaneous
griffonilide[b] (**2**)	6.62 dd (9.5, 2.5)	6.27 dd (9.5, 1.9)	4.33 dt (7.6, 2.5, 1.9)	3.53 dd (10.8, 7.6)	4.90 dd (10.8, 1.9)	5.89 d (~2)	
griffonilide dibenzoate[c] (**3**)	6.77 dd (9.5, 1.9)	6.32 dd (9.5, 1.9)	6.09 dt (8.3, 1.9, 1.9)	5.76 dd (10.8, 8.3)	5.24 dd (10.8, 1.9)	6.07 d (~2)	ArH 7.3–7.7 (6H,m) 7.9–8.1 (4H,m)
dihydro-griffonilide[b] (7)	1.9–2.2 (1H,m)[d] 2.6–2.9 (1H,m)	1.1–1.6 (1H,m)[d] 2.3–2.6 (1H,m)	3.60 m (11.2, 9.1, 4.5)	3.08 t (9.1, 9.1)	4.67 dd (9.1, 1.6)	5.77 t (1.6, 1.6)	
dihydro-griffonilide dibenzoate[c]	2.3–2.8 (1 of 2H,m)[d] 2.9–3.2 (1H,m)	2.3–2.8 (1 of 2H,m)[d] 1.5–2.0 (1H,m)	5.3–5.6 (1 of 2H,m)	5.3–5.6 (1 of 2H,m)	5.05 brd (~8)	5.98 brs	ArH 7.2–7.6 (6H,m) 7.8–8.1 (4H,m)
dasycarponilide[b] (**6**)	6.69 d (9.5)	6.41 dd (9.5, 5.4)	4.39 dd (5.4, 4.1)	3.63 dd (10.5, 4.1)	5.22 dd (10.5, 1.9)	5.90 d (1.9)	
dasycarponilide dibenzoate[c] (**11**)	6.88 d (9.5)	6.51 dd (9.5, 5.0)	6.16 dd (5.0, 4.4)	5.40 dd (11, 4.4)	5.65 dd (11, 1.9)	6.07 d (1.9)	ArH 7.3–7.7 (6H,m) 7.9–8.2 (4H,m)
dihydrodasycarponilide[b] (**8**)	2.55 (1H, brd) 2.67 (1H, brd)	1.2–1.7 (1H,m) 1.9–2.2 (1H,m)	4.00 m (2.8, ~2, ~2)	3.30 dd (9.2, 2.8)	4.96 brd (9.2, ~1)	5.75 brs (~1)	
dihydrodasycarponilide dibenzoate[c]	mixture of multiplets between 1.5–3.0		5.90 m (3 x ~2.5–2.8)	5.10 dd (10.2, 2.5)	5.44 brd (10.2)	5.99 brs	ArH 7.3–7.7 (6H,m) 7.9–8.2 (4H,m)

[a]Spectra were determined in stated solvent at 90 MHz with Me_4Si as internal standard. Chemical shifts (δ) in ppm, coupling constants (J) in Hz are given in parentheses, and multiplicities are designed by the following symbols: s=singlet, d=doublet, m=multiplet, t=triplet, and br=broadened signal.

[b]In MeOH d_4.

[c]In $CDCl_3$.

[d]Assignments for H-2 and H-3 are not firm.

and less intense than for lithospermoside and indicating a significant change in the chirality of the $\alpha,\beta,\gamma,\delta$-unsaturated nitrile system.

The structure of dasycarponin was determined from studies on the modified aglycone, dasycarponilide (**6**). Its ^{13}C-nmr spectrum (table 3) is close to that of

TABLE 3. Carbon nuclear magnetic resonance peaks for griffonilide (**2**) and dasycarponilide (**6**).[a]

Carbon	Griffonilide		Dasycarponilide	
	δ_c	$^1J_{CH}$	δ_c	$^1J_{CH}$
1	164.7 s		164.3 s	
2	120.6	168.4	123.3	169.1
3	144.2	164.7	139.7	165.5
4	73.6	139.7	68.7	154.4
5	80.0	142.7	74.4	141.9
6	85.1	153.0	83.4	155.9
7	112.5	182.4	113.3	182.4
8	175.8 s		175.8 s	

[a]Spectra were taken in MeOH-d_4 at ambient temperature (20–25°). Chemical shifts are in ppm relative to tetramethylsilane as internal standard under broad band decoupling. Coupling constants were measured from spectra taken under undecoupled-NOE enhanced conditions. Chemical shift values followed by s are singlets and unmarked are doublets for peaks determined under off-reasonance conditions.

griffonilide, with major changes in the chemical shift for C–3, C–4 and C–5 and in the $^1J_{CH}$ coupling constant for C–4. The carbon peaks were designated by single frequency decoupling experiments, the data being presented in a graphical form for interpretation of results according to Birdsall *et al.*, (8). The ^{1}H-nmr peaks of dasycarponilide (**6**) (table 2) were assigned by comparison with that of griffonilide and by double irradiation experiments. All protons for the two modified aglycones have the same relative chemical shift. However, one change must be made for the reported assignments of the griffonilide peaks. The H–3 proton of dasycarponilide (**6**) must be located at δ 6.41 to accomodate the large coupling constant of 5.4 Hz, which could not be due to allylic coupling since these are less than 3.5 Hz (9), but is not unusual for vinylic coupling (10). Accordingly, the published assignments (3) for H–2 and H–3 of griffonilide, made at a time when a clear choice was not available, should now be reversed.

The reasonable assumption was made that dasycarponilide (**6**) was a diastereomer of griffonilide (**2**) and that the proton spin coupling values could be used to assign stereochemistry. For this, a reference point was needed. Although the allylic coupling constant associated with the lactone in both aglycones is identical ($^4J_{6,7}$=1.9 Hz), suggesting identical stereochemistry for that unit, the cd curves (figures 1 and 3) are distinctly different. That this difference was a result of the overall chirality change involving the $\alpha,\beta,\gamma,\delta$-unsaturated γ-lactone system was shown by the nearly identical circular dichroism curves (see Experimental) obtained for the 2,3-dihydro- derivatives **7** and **8**. Dihydrogriffonilide (**7**) and dihydrodasycarponilide (**8**) showed molecular ellipticities of $[\theta]_{235}$ −9,000 ($\Delta\epsilon$ −2.7) and $[\theta]_{237}$ −11,900 ($\Delta\epsilon$ −3.6), respectively. The electronic transition of the unsaturated lactone associated with these maxima must be the $\pi\rightarrow\pi^*$, since

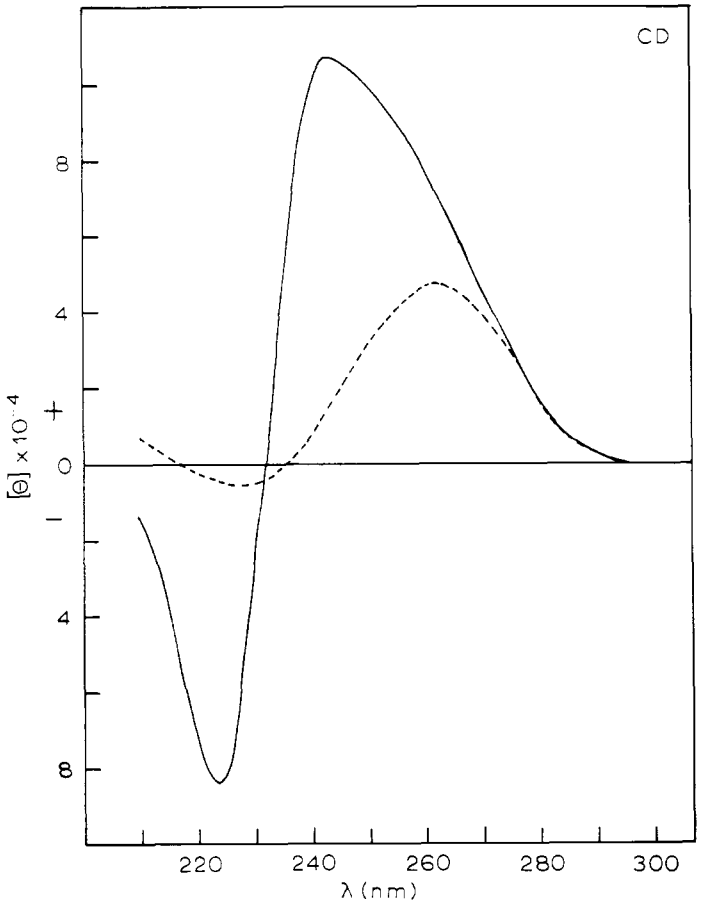
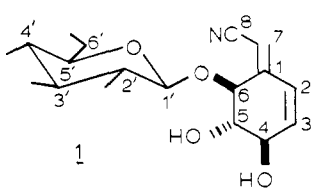


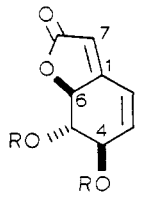
FIG. 3. Circular dichroism curves for dasycarponilide (**6**) (- - -) and dasycarponilide dibenzoate (**11**) (———).

dihydrodasycarponilide (**8**), in addition, exhibited a very weak positive maximum $[\theta]$ +14 ($\Delta\epsilon$ +0.004) at 290 nm assignable to the n→π^* transition for the lactone carbonyl (11). The *S*-configuration at C-6, that follows from use of the lactone $\pi\rightarrow\pi^*$ circular dichroism (11), provides additional support for the absolute stereochemistry of griffonilide (**2**) as established from the dibenzoate rule (5). Furthermore, the positive n→π^* cd observed for dihydrodasycarponilide (**8**) is also in agreement with the lactone stereochemistry rule of Beecham (12). Apparently, the difference in the cd curves between griffonilide (**2**) and dasycarponilide (**6**) (figure 1 and 3) not observed for the dihydro- derivatives **7** and **8** results from a strong and overriding "allylic" oxygen effect (12,13,14) caused by the ϵ-substituent of the $\alpha,\beta,\gamma,\delta$-unsaturated γ'-lactone exerted via extended conjugation. This would suggest a stereochemical difference at C-4 between the two aglycones. Pertinent examples from the literature are shikimic and 5-epishikimic acids, which are epimeric at the allylic position and show opposite cd curves, $[\theta]_{255}$ +3,600 and $[\theta]_{252}$ −13,500, respectively (15).

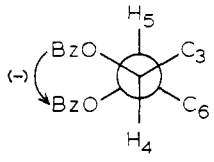
With the lactone chirality established, and only the configuration at C-4 and



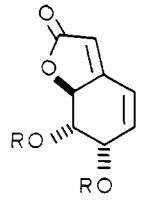
1



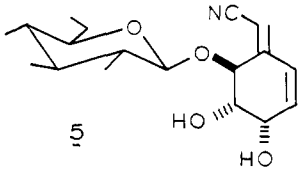
2; R = H
3; R = C_6H_5CO
7; R = H, 2,3-dihydro



4



6; R = H
8; R = H, 2,3-dihydro
11; R = C_6H_5CO



5

C–5 remaining, the conformation of the six-membered ring of dasycarponilide (**6**) must be known before the proton coupling values can be utilized for assignment of stereochemistry. Theoretically, the two extreme conformations are the half-chair and the boat as shown in **9** and **10**, respectively. Since dasycarponilide dibenzoate (**11**) gives a cd curve (figure 3) with a positive Cotton effect at the higher wavelength ($[\theta]_{243}$ +107,000 and $[\theta]_{223}$ −83,700), the dibenzoyl groups must be disposed in a clockwise manner (5) and opposite to that drawn in **4**. Dreiding models of the three possible diastereomers, 4*S*, 5*S* (**12**), 4*R*, 5*R* (**13**), and 4*S*, 5*R* (**14**) (the latter in the boat form, to avoid the energetically unfavored *trans*-diaxially-substituted half-chair conformer) show that all would give the clockwise-ordered 1,2-dibenzoates. The boat forms of **12** and **13**, on the other hand, would result in counterclockwise dibenzoates, incompatible with the cd results. Dibenzoates of dihydrogriffonilide (**7**) and dihydrodasycarponilide (**8**) gave cd curves of the same order as observed for the dehydro-compounds.

The dihedral angles between the protons in structures **12**, **13** and **14** were measured from Dreiding models and are given in table 4. From these values, coupling constants were calculated using modified Karplus equations. For ^3J involving only sp^3 carbons (H4–H5 and H5–H6), the equation devised for carbohydrates was employed (16), while vinylic (^3J) and allylic (^4J) couplings were obtained according to Garbisch (10). In spite of the known inaccuracies of the Karplus-type equations, in providing an exact relationship between the coupling constant and dihedral angle, they can be of value when their results are used to differentiate between structures with widely varying dihedral angles. Comparison of the calculated coupling values (table 4) for structures **12**, **13** and **14** with

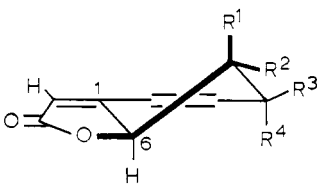
TABLE 4. Calculated dihedral angles (ϕ) and coupling constants (J) for possible structures of dasycarponilide and observed coupling constants.[a]

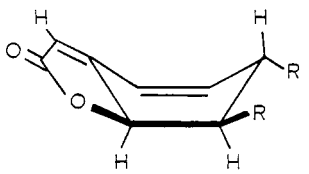
Protons Coupled	Calculated Values for Structures						Observed Values for Dasycarponilide
	4*S*,5*S* (**12**)		4*R*,5*R* (**13**)		4*S*,4*R* (**14**)		
	ϕ(deg)	J (Hz)	ϕ(deg)	J (Hz)	ϕ(deg)	J (Hz)	J (Hz)
H_2–H_4	140	0.3	100	2.5	75	2.4	0
H_3–H_4	40	4.9	75	2.9	105	3.2	5.4
H_4–H_5	50	3.6	55	2.8	160	8.9	4.1
H_5–H_6	180	10.1	55	2.8	10	8.7	10.5
H_6–H_7	110	2.1	110	2.1	110	2.1	1.9

[a]The dihedral angles were estimated from Dreiding models and are $\pm 5°$. See the text for the method of calculating coupling constants.

those observed for dasycarponilide shows the best agreement is with structure **12**, the C–4 epimer of griffonilide (**2**). Wide discrepancies occur for the others. A literature example, conduritol (3,4,5,6-tetrahydroxycyclohex-1-ene) isomer F (**15**), has both pseudoequatorial and pseudoaxial allylic protons which are reasonable models for the H–4 protons of griffonilide and dasycarponilide (17). The pseudoaxial proton has vinylic and allylic coupling constants of 1.9 and 2.1 Hz, comparable to griffonilide (**2**) values of 1.9 and 2.5 Hz, respectively; the pseudoequatorial proton has 5.3 and 0.5 Hz, in good agreement with 5.4 and 0 Hz for dasycarponilide (**6**).

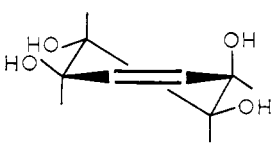
The data presented supports the conformational structure of dasycarponilide as **12** and the molecular structure as **6**. Therefore, dasycarponin should be represented as **5** and is the C–4 epimer of lithospermoside (**1**). Attempts at selectively oxidizing the allylic alcohol of both glycosides to a common ketone failed, and similar attempts with the modified aglycones **2** and **6**, were likewise unsuccessful.





9; $R^1 = R^2 = R^3 = R^4 = H$
12; $R^1 = R^3 = H$, $R^2 = R^4 = OH$
13; $R^1 = R^3 = OH$, $R^2 = R^4 = H$

10; R = H
14; R = OH



15

Thus, a chemical interrelationship between the two was not realized. In addition to the two glucosides reported here, the literature records two other cyano-containing glucosides of related structure, simmondsin from seeds of the jojoba plant (*Simmondsia californica*) (18) and menisdaurin from vines of *Menispermum dauricum* DC (19).

EXPERIMENTAL[2]

PLANT MATERIAL.—*Thalictrum rugosum* Ait., and *T. revolutum* DC. plants were grown in the Medicinal Plant Garden of the College of Pharmacy, Ohio State University. *T. dasycarpum* Fisch. and Lall. were collected by Dr. E. M. Herrick, Twinsburg, Ohio. Voucher specimens are on file.

ISOLATION OF LITHOSPERMOSIDE (**1**).—Air-dried powdered roots (14.1 kg) of *T. rugosum* were extracted by percolation with ethanol. During concentration of the extract, a precipitate (7.0 g) formed which was collected and crystallized from methanol-water (1:2). After several recrystallizations, 1.78 g of fine white needles were formed; mp 272–4°(d), $[\alpha]^{27}D-138°$ (*c* 0.5, H_2O); cd (c 3.0 x 10^{-3}, H_2O) 25° $[\theta]_{295}$ 0, $[\theta]_{260}-59{,}400$, $[\theta]_{241}$ 0, $[\theta]_{228}+34{,}700$; uv λ max 259 nm (log ε 4.18); ir ν max 2220 cm^{-1} (CN) ^{1}H-nmr (90 MHz, D_2O) δ 6.33 (1H, H-2, dd, *J* 10,1), 6.12 (1H, H-3, dd, *J* 10,3), 5.61 (1H, H-7, d, *J*~1), 4.28 (1H, H-4, m) and remaining protons of aglycone and glucose between 4.7–4.9 and 3.3–4.1. ^{13}C-nmr peaks are given in table 1. Literature values for lithospermoside (2) are mp 278–9° and $[\alpha]^{20}D-156°$ (H_2O) and for griffonin (3) mp. 263–5° and $[\alpha]^{22}D-149°$ (H_2O).[4] Ms (ei) peaks were observed at *m/e* (%): 330 (1, MH^+), 329 (0.1, M^+), 268 (0.7), 196 (2), 178 (2), 168 (3), 150 (17), 149 (100), 132 (10), 131 (14), 123 (11), 122 (17), 103 (38), 73 (43), 60 (27), 57 (13), and 43 (23). Authentic samples of lithospermoside and griffonin were compared directly with the *T. rugosum* glycoside by mixture mp, by cd, ir and ^{1}H-nmr spectra and by tlc. Tlc on silica gel G with chloroform-methanol (3:1) gave R_f 0.24 after spraying with diethyl ether-sulfuric acid (10:1) and heating at 110° for several minutes. In addition, griffonin was also compared by uv and ^{13}C-nmr spectra.

Anal. Calcd. for $C_{14}H_{19}NO_8$ (MW 329): C, 51.06; H, 5.82; N, 4.25.
Found: C, 51.11; H, 5.81; N, 4.23%.

LITHOSPERMOSIDE HEXAACETATE.—Lithospermoside (**1**, 60 mg) was treated with 1.5 ml each of acetic anhydride and pyridine at room temperature for 8 hrs. After the usual workup, the amorphous acetate (77 mg) homogeneous by tlc (R_f 0.89 on silica gel G with $CHCl_3$) had the following physical properties: $[\alpha]^{22}D-108°$ (*c* 1.35, $CHCl_3$); uv λ max 252 nm (log ε 4.47); ir ($CHCl_3$) ν max 2220 (CN) and 1760 (acetate) cm^{-1}; ^{1}H-nmr (90 MHz, $CDCl_3$) δ 2.00, 2.03, 2.05, 2.09, 2.10 and 2.12 (6s, 6 OAc); and ^{13}C-nmr (22.63 MHz, $CDCl_3$) δ_c 170.6, 170.3(2x), 169.2(2x), 169.0, 150.8, 131.3, 128.8, 115.7, 102.0, 100.4, 74.1, 72.9, 72.3, 71.7, 70.9, 69.0, 68.4, 61.5, 20.8(3x) and 20.5(3x). Literature value (3) $[\alpha]D-112.75°$ ($CHCl_3$).[5]

ENZYMATIC HYDROLYSIS OF LITHOSPERMOSIDE (**1**).—A reaction mixture of 0.5 g lithospermoside, 25 mg emulsin and 6.5 ml water was kept at room temperature for 14 days and then passed through a Sephadex™ LH-20 column (30 g). The column was eluted with water, and a 5 ml fraction was collected. The residues left after evaporation of the solvent were monitored by tlc using silica gel G and chloroform-methanol (3:1). Fractions 10 and 11 gave 31 mg of glucose (R_f 0.1 on tlc) identified by cochromatography with a known sample on paper [Whatman #1, ethyl acetate-pyridine-water (12:5:4)] and by the mutarotation equilibrium $[\alpha]D+52°$ (20). Fractions 12–15 yielded 305 mg of starting material.

Fractions 16–18 afforded 42 mg of griffonilide (**2**) [tlc R_f 0.19 on silica gel G and chloroform-methanol (12:1) developed twice] which crystallized from methanol: mp 184–5° (lit. value (4) mp 184–5); $[\alpha]D-14°$ (*c* 0.014, MeOH); cd (*C* 8.3 x 10^{-4}, MeOH) 25° $[\theta]_{300}$ 0, $[\theta]_{278}-6{,}300$, $[\theta]_{263}$ 0, $[\theta]_{246}+6{,}600$; ms (ci, i-butane) *m/e* 169 (100%, M+1, $C_8H_8O_4$ requires 168); and ir (KBr) ν max 1725 (lactone) and 1635 (olefin) cm^{-1}. The ^{1}H- and ^{13}C-nmr peaks are given in tables 2 and 3, respectively. The spectral properties were comparable to those reported for griffonilide (3).

---

[3]Melting points are uncorrected. Nmr spectra were determined in stated solvents with tetramethylsilane as internal standard on Varian A-60A or Bruker HX-90E instruments, the latter equipped for Fourier transform analysis. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. Ir spectra were taken in KBr windows on a Beckman IR 4230. Uv spectra were taken in methanol on a Cary 15 instrument. Mass spectra were obtained on an AEI MS-9 or DuPont 21-491 instruments by direct inlet probe at 70eV. Optical rotations were measured on a Perkin-Elmer 241 photoelectric polarimeter and cd spectra in methanol on a Durrum-Jasco ORD/UV-5 spectropolarimeter with Sproul Scientific SS-20 modification. Microanalysis was by Scandinavian Microanalytical Laboratory, Herlev, Denmark.

[4]The authors have informed us that the specific rotation of +6° reported in their paper is in error and should be replaced by the new value given here.

[5]The authors have informed us that the minus sign was inadvertantly omitted from the value reported.

ACID HYDROLYSIS OF LITHOSPERMOSIDE (**1**).—A 0.35 g sample of the glycoside was suspended in 3.5 ml methanol and 7 ml of 1*N* HCl added. After heating on a steam bath for 2 hrs, the reaction mixture was evaporated to dryness, and the residue was separated on a column of silicic acid (10 g). Elution with 6% methanol in chloroform gave 70 mg of griffonilide (**2**). Unreacted lithospermoside (103 mg) was obtained with 40% methanol in chloroform, followed by 120 mg of glucose with 50% methanol in chloroform.

GRIFFONILIDE DIBENZOATE (**3**).—Griffonilide (**2**, 15 mg) was reacted with 0.09 ml of benzoyl chloride in 1.5 ml of pyridine at room temperature for 24 hrs. The mixture was evaporated to dryness and the residue passed through a column of silicic acid (1.5 g) with chloroform as eluant. The eluted dibenzoate **3** (23.5 mg) crystallized from ethanol as colorless needles: mp 195–6°; $[\alpha]^{26}$D −194° (*c* 0.1, MeOH); cd (C 2.7 x 10^{-3}, MeOH) 25° $[\theta]_{300}$ 0, $[\theta]_{276}$ +8,500, $[\theta]_{237}$ −133,000, $[\theta]_{225}$ 0 and $[\theta]_{222}$ +63,900; uv λ max 234 nm (log ε 4.14) and 252 (shld, 4.04); ms (ei) *m/e* (%) 376 (0.08, M^-), 254 (4, M-$C_6H_5CO_2H$), 122 (1, $C_6H_5CO_2H$), 111 (13), 105 (100, C_6H_5CO), 77 (38), and 51 (13). The ^{1}H-nmr peaks are found in table 2.

Anal. Calcd for $C_{22}H_{16}O_6$ (MW 376): C, 70.21; H, 4.29.
Found: C, 69.85; H, 4.34%.

DIHYDROGRIFFONILIDE (**7**).—Griffonilide (**2**, 10 mg) in 6.5 ml of methanol was hydrogenated with 5 mg of palladium on charcoal as catalyst. After one equivalent of hydrogen was consumed (7 min), the reaction was stopped and the mixture filtered. Evaporation of solvent at reduced pressure gave 10 mg of dihydrogriffonilide (**7**) as a colorless residue, homogeneous by tlc, R_f 0.37 on silica gel G and chloroform-methanol (10:1), and possessing the following physical properties: uv λ max 211 nm (log ε 4.09); cd (C 1.3 x 10^{-2}, MeOH), 25° $[\theta]_{275}$ 0 and $[\theta]_{235}$ −9,000; ms (ei) *m/e* 170.0585 (4%, M^-) $C_8H_{10}O_4$ requires 170.0579; and ^{1}H-nmr peaks as in table 2.

DIHYDROGRIFFONILIDE DIBENZOATE.—Dihydrogriffonilide (**7**, 4 mg) was treated with 0.01 ml of benzoyl chloride in 0.2 ml of pyridine at room temperature for 16 hrs then mixed with 20 ml of chloroform, and the solution was extracted with water (2 x 20 ml). Evaporation of the solvent at reduced pressure gave a crystalline solid that recrystallized from ethanol as colorless fine needles (4.6 mg); mp 204–6°; cd (C 1.7 x 10^{-3} M, MeOH) 25° $[\theta]_{290}$ 0, $[\theta]_{250}$ +10,000, $[\theta]_{237}$ −27,000, $[\theta]_{232}$ 0, $[\theta]_{224}$ +54,000, and $[\theta]_{217}$ 0; uv λ max 266 nm (log ε 4.63) and 228 (4.96); ir ($CHCl_3$) ν max 1730 cm^{-1} (benzoate); ms (ei) *m/e* (%) 378.1111 (3, M^-) $C_{22}H_{18}O_6$ requires 378.1103, 256 (9, M-$C_6H_5CO_2H$), 134 (11, M-2$C_6H_5CO_2H$), 121 ($C_6H_5CO_2$), 105 (100, C_6H_5CO), and 77 (28, C_6H_5); and ^{1}H-nmr peaks as in table 2.

ISOLATION OF DASYCARPONIN (**5**).—The ethanolic extract from the percolation of 3.46 kg of dried powdered roots of *T. dasycarpum* deposited 18.6 g of a precipitate upon concentration by evaporation at reduced pressure. Repeat crystallization of the precipitate from methanol-water (3:1) gave 3.13 g of dasycarponin (**5**) as colorless rhombic crystals: mp 253–5°(d); $[\alpha]^{27}$D −11° (*c* 0.25, H_2O); cd (C 7.6 x 10^{-3}, H_2O) 25° $[\theta]_{295}$ 0, $[\theta]_{277}$ +4,610, $[\theta]_{258}$ 0, $[\theta]_{230}$ −23,000; uv λ max 261 nm (log ε 4.17); ir (KBr) ν max 3600–3100 (broad), 3040, 2980, 2930, 2890, 2850, 2230, 1633, 1598, 1460, 1407, 1375, 1339, 1170, 1120, 1102, 1063, 1040, 995, 893, and 860 cm^{-1}; ^{1}H-nmr (90 MHz, D_2O) δ 6.35 (1H, H-2, brd, *J* 10.5), 6.05 (1H, H-3, brd, *J* 10.5), 5.70 (1H, H-7, s), 4.28 (1H, H-4, m), and remaining protons of aglycone and glucose between 3.1–4.0 and 4.6–5.0; ^{13}C-nmr peaks in table 1; ms (ei) *m/e* (%) 329 (0.9, M^+) and 149 (100, M-$C_6H_{12}O_6$); and ms (ci, i-butane) 330 (7, MH^-) and 150 (100, MH-$C_6H_{12}O_6$). Tlc showed R_f 0.19 on silica gel G with chloroform-methanol (3:1).

Anal. Calcd for $C_{14}H_{19}NO_8$: C, 51.06; H, 5.82; N, 4.25.
Found: C, 51.11; H, 5.87; N, 4.39%.

ENZYMATIC HYDROLYSIS OF DASYCARPONIN (*5*).—Dasycarponin (120 mg) and emulsion (50 mg) in 10 ml of water were incubated at room temperature for 10 days. The filtrate was passed through a 30 g column of Sephadex™ LH-20 with water as eluant. Column fractions of 5 ml were evaporated to dryness and monitored by tlc on silica gel G with chloroform-methanol (3:1).

Fractions 15–17 gave the aglycone, dasycarponilide (**6**), 40 mg, and tlc R_f 0.23 on silica gel G with chloroform-methanol (12:1), developed twice. Crystallization was from chloroform-methanol (1:1): mp 164–5°; $[\alpha]^{27}$D +370° (*c* 0.01, MeOH); cd (C 6.0 x 10^{-4}, MeOH) 25° $[\theta]_{300}$ 0, $[\theta]_{261}$ +48,000, $[\theta]_{235}$ 0, $[\theta]_{226}$ −5,000, and $[\theta]_{215}$ 0; uv λ max 252 nm (log ε 4.13); ir (KBr) ν max 3600–3100, 2910, 1725, 1637, 1430, 1175, 1155, 1113, 1080, 1036, 910, 865, 847, 817, 795 and 737; ms (ei) *m/e* 168.0425 (23, M^-, $C_8H_8O_4$ requires 168.0422), 150 (31, M-H_2O), 139 (62), 121 (100), 111 (63), 94 (30), 83 (26), 81 (24) and 67 (14); ms (ci, i-butane) 169 (100, MH^+), 151 (10, MH-H_2O), and 123 (8); ^{1}H-nmr peaks in table 2; and ^{13}C-nmr in table 3.

DASYCARPONIN HEXAACETATE.—Dasycarponin (**5**, 0.1 g) in 3.5 ml pyridine and 2.5 ml acetic anhydride was stirred for 8 hrs at room temperature, then 3 ml water was added. The reaction mixture was extracted with diethyl ether (20 ml x 4), and the combined ether extract was washed successively with 10% aq. $NaHCO_3$, 10% aq. Hcl and water. On evaporation of the dried (Na_2SO_4) ether layer, 164 mg of dasycarponin hexaacetate was obtained, as an amorphous

solid: $[\alpha]^{25}D+20°$ (*c* 0.25, $CHCl_3$); uv λ max 253 nm (log ε 4.21); ir ($CHCl_3$) ν max 2215 (CN) and 1765 (acetate) cm^{-1}; ^1H-nmr (90 MHz, $CDCl_3$) δ 2.00, 2.02, 2.04, 2.06, 2.08 and 2.15 (6s, 6 OAC); ^{13}C-nmr (22.63 MHz, $CDCl_3$) δ_c 170.5, 170.0, 169.7, 169.6(2x), 169.3, 150.3, 133.0, 127.3, 116.0, 102.2, 101.2, 75.2, 72.7, 72.5, 71.0, 68.2, 68.0, 66.8, 61.4, 20.7(3x) and 20.5(3x); ms (ei) *m/e* (%) 581 (0.7, M^+, $C_{26}H_{31}NO_{14}$ requires 581) and 83 (100); and ms (ci, i-butane) 582 (37, MH^+) and 331 (100).

ACID HYDROLYSIS OF DASYCARPONIN (**5**).—A 0.35 g sample of dasycarponin was dissolved in 5 ml methanol-water (1:9), 2 ml of 2*N* HCl was added, and the mixture heated on the steam bath for 2.5 hrs. The residue remaining after evaporation of the solvent was mixed with methanol, and the insoluble material (dasycarponin, 45 mg) was collected by filtration. The filtrate was evaporated, and the residue was chromatographed on 12 g of silicic acid with chloroform and chloroform with increasing amounts of methanol as eluants. The 8% methanol in chloroform effluent gave 58 mg of dasycarponilide (**6**), and the 40% methanol in chloroform afforded 102 mg of *D*-glucose identified by paper chromatographic comparison with an authentic sample and by its equilibrium specific rotation.

DASYCARPONILIDE DIBENZOATE (**11**).—Dasycarponilide (**6**, 30 mg) was treated with 1 ml pyridine and 0.06 ml benzoyl chloride for 24 hrs. The mixture was evaporated to dryness, and the residue was chromatographed on 1 g of silicic acid with chloroform to give a fraction that crystallized from ethanol as colorless crystals (49 mg): mp 105–8°; $[\alpha]^{25}D+367°$ (*c* 0.1, MeOH); cd (C 2.7 x 10^{-3}, MeOH) 25° $[\theta]_{300}$ 0, $[\theta]_{243}+107{,}000$, $[\theta]_{232}$ 0 and $[\theta]_{223}-83{,}700$ uv λ max 228 nm (log ε 4.29), and 256 (shld, 3.69); ms (ei) *m/e* (%) 376 (0.3, M^+), 254 (2), 122 (37), 105 (100), 77 (49) and 51 (17); and ^1H-nmr peaks as given in table 2.

Anal. Calcd for $C_{22}H_{16}O_6$ (MW 376): C, 70.21; H, 4.29. $C_{22}H_6O_{16}0.5C_2H_5OH$: C, 69.16; H, 4.79.
Found: C, 69.23; H, 4.70%.

DIHYDRODASYCARPONILIDE (**8**).—Dasycarponilide (**6**, 10 mg) in 6.5 ml methanol was hydrogenated over 5% palladium on charcoal as catalyst. After one equivalent of hydrogen was consumed (12 min), the reaction was stopped. Removal of the catalyst by filtration and evaporation of the solvent left 11 mg of a crystalline residue of the dihydro-product **8**, tlc R_f 0.44 with chloroform-methanol (10:1): mp 127–9°; uv λ max 216 nm (log ε 3.97); cd (C 6.1 x 10^{-3}, MeOH) 25° $[\theta]_{300}$ 0, $[\theta]_{290}+14$, $[\theta]_{278}$ 0 and $[\theta]_{237}-11{,}900$; ms (ei) *m/e* 170.0585 (3%, M^+) $C_8H_{10}O_4$ requires 170.0579; and ^1H-nmr peaks as in table 2.

DIHYDRODASYCARPONILIDE DIBENZOATE.—Dihydrodasycarponilide (**8**, 2 mg) was treated with 0.01 ml benzoyl chloride in 0.2 ml pyridine for 16 hrs at room temperature with stirring. The mixture was taken up in 20 ml chloroform and extracted with water (20 ml x 2); the chloroformic solution evaporated to dryness. The colorless residue (2.1 mg) gave a cd curve (C 7.7 x 10^{-3}, MeOH) 25° $[\theta]_{270}$ 0, $[\theta]_{230}+59{,}300$, $[\theta]_{218}$ 0 and $[\theta]_{212}-20{,}200$; uv λ max 272 nm (log ε 3.84) and 225 (4.88); ir ($CHCl_3$) ν max 1730 cm^{-1} (benzoate); and ^1H-nmr peaks as in table 2.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service Research Grant HL-07052 from the National Institutes of Health. We thank Dr. W.-N. Wu for a supply of lithospermoside, Mr. C. Weisenberger of the Chemistry Department for the mass spectra, and Mr. J. Fowble for some ^{13}C-nmr studies. We are grateful to Prof. P. L. Schiff, Jr., for a sample and spectra of griffonin, and to Prof. R. Wylde for a sample of lithospermoside.

Received 24 April 1979.

LITERATURE CITED

1. D. H. Lee, 1968. *Novel Cyanides from Thalictrum Species*. Ph.D. Thesis Part II. University of the West Indies. pp. 87–145.
2. A. Sosa, F. Winternitz, R. Wylde and A. A. Pavia, *Phytochemistry*, **16**, 707 (1977).
3. D. Dwuma-Badu, W. H. Watson, E. M. Gopalakrishna, T. U. Okarter, J. E. Knapp, P. L. Schiff, Jr. and D. J. Slatkin, *Lloydia*, **39**, 385 (1976).
4. E. M. Gopalakrishna, W. H. Watson, D. Dwuma-Badu, T. U. Okarter, J. E. Knapp, P. L. Schiff, Jr. and D. J. Slatkin, *Cryst. Struct. Comm.*, **5**, 779 (1976).
5. N. Harada and K. Nakanishi, *J. Am. Chem. Soc.*, **91**, **3989** (1969).
6. N. Harada and K. Nakanishi, *Acc. Chem. Res.*, **5**, 257 (1972).
7. T. E. Walker, R. E. London, T. W. Whaley, R. Barker, and N. A. Matwiyoff, *J. Am. Chem. Soc.*, **98**, 5807 (1976).
8. B. Birdsall, N. J. M. Birdsall, and J. Feeney, *J. C. S. Chem. Commun.*, 316 (1972).
9. M. Barfield, R. J. Spear, and S. Sternhell, *Chem. Rev.*, **76**, 593 (1976).
10. E. D. Garbisch, *J. Am. Chem. Soc.*, **86**, 5561 (1964).
11. I. Uchida and K. Kuriyama, *Tetrahedron Lett.*, **3761** (1974).
12. A. F. Beecham, *Tetrahedron*, **28**, 5543 (1972).

13. A. F. Beecham, A. McL. Mathieson, S. R. Johns, J. A. Lamberton, A. A. Sioumis, T. J. Batterham and I. G. Young, *Tetrahedron*, **27**, 3725 (1971).
14. A. F. Beecham, *Tetrahedron*, **27**, 5207 (1971).
15. U. Weiss and H. Ziffer, *J. Org. Chem.*, **28**, 1248 (1963).
16. R. J. Abraham, L. D. Hall, L. Hough and K. A. McLauchlan, *J. Chem. Soc.*, 3699 (1962).
17. R. J. Abraham, H. Gottschalck, H. Paulsen and W. A. Thomas, *J. Chem. Soc.*, 6268 (1965)
18. C. A. Elliger, A. C. Waiss, jun., and R. E. Lundin, *J. C. S. Perkin I*, 2209 (1973).
19. K. Takahashi, S. Matsuzawa and M. Takani, *Chem. Pharm. Bull.* (Tokyo), **26**, 1677 (1978).
20. W. W. Pigman and R. M. Goepp, Jr., 1948. *Chemistry of the Carbohydrates*, Academic Press, New York. p. 63.