Phytochemistry, 1977, Vol. 16, pp 707–709. Pergamon Press. Printed in England.

STRUCTURE OF A CYANOGLUCOSIDE OF *LITHOSPERMUM PURPUREO-CAERULEUM*

A. SOSA*, F. WINTERNITZ*, R. WYLDE* and A. A. PAVIA†

*Laboratoire de produits naturels et biochime organique de synthèse Equipe de Recherches nr. 195 du C.N.R.S. associée à l'E. N.S.C.M. Ecole Nationale Supérieure de Chimie de Montpellier 8, rue de l'Ecole Normale, 34075 Montpellier, France; †Laboratoire de Chimie II. Faculté des Sciences d'Avignon 33, rue Louis Pasteur 84000 Avignon, France

(*Received* 24 *May* 1976)

Key Word Index—*Lithospermum officinale*; *L. purpureo-caeruleum*; Boraginaceae; cyanomethylenecyclohexenyl glucoside.

Abstract—A new glucoside has been isolated from roots of *Lithospermum officinale* and *L. caeruleum*. It is not cyanogenetic although containing nitrile group in the aglycone moiety. Its structure has been elucidated by ^{1}H and ^{13}C NMR spectroscopy as 6-*O*-β-D-glucopyranosyl-1-cyanomethylene-4,5-dihydroxy-2-cyclohexene.

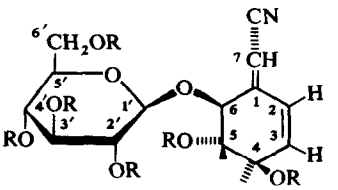
INTRODUCTION

The report, in 1941, that Nevada Indians used infusions of *Lithospermum ruderale* L. as a contraceptive drug [1] has stimulated intensive studies on the chemical constituents of several species of *Lithospermum* [2–7]. The physiological activity of the plant is ascribed [3] to its phenolic constituents, particularly to lithospermic acid whose structure has been recently established [4, 5]. But, although the acidic constituents are well known, the carbohydrate constituents have received little attention.

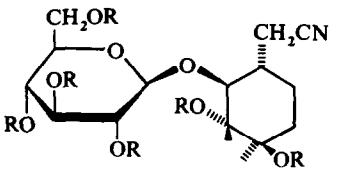
In 1955, one of us [6] described the isolation of a new, unknown glycoside, lithospermoside, from the extracts of *L. purpureo-caeruleum* roots. This compound was present in the plant together with sugars from which it was separated by partition chromatography. It is, probably, the same substance as isolated by Gorman *et al.* [7] one year later. Owing to difficulties of isolation and the lack of modern spectroscopic methods its structure could not be elucidated at that time. We have now identified it by NMR procedures as a new cyanoglucoside.

RESULTS AND DISCUSSION

Both elemental analysis and MS of (1) indicated the empirical formula $C_{14}H_{19}NO_8$. The IR spectrum showed broad adsorption at 3000–3200 cm^{-1} (ν–OH), together with a very sharp and strong band at 2200 cm^{-1} (ν–CN) which is to be expected for a conjugated nitrile group. Comparison of these data with other cyanoglucosides [8,9] shows that (1) is different from both gynocardin or triglochin. In fact (1) is not cyanogenetic since no HCN can be detected upon enzymatic or acid



1 R = H 2 R = Ac



3 R = H 4 R = Ac

hydrolysis. The UV spectrum exhibited strong absorption at λ_{max} 260 nm (ε, 21400) in good agreement with a cyclohexadiene structure but PMR and ^{13}C NMR spectroscopy showed conclusively that it is: 6-*O*-β-D-glucopyranosyl-1-cyanomethylene-4,5-dihydroxy-2-cyclohexene(1).

The PMR spectrum of (1) displayed three downfield signals corresponding to three olefinic protons respectively at 6.37 ppm (H_2) 6.12 ppm (H_3) and 5.63 ppm (H_7). Signals at 6.37 and 6.12 ppm represent the AB part of an ABX system. Double resonance experiments at 100 and 250 MHz have shown the proton X, namely H_4, to be located at 4.30 ppm. On the other hand, double irradiation of proton 7 results in sharpening each component of the AB part. The long-range coupling between proton 7 and both protons 2 and 3 is estimated to be $J_{2,7} \simeq J_{3,7} \simeq 0.5$ Hz. PMR analysis of signals at 6.37 and 6.12 ppm gives the following coupling constants: $J_{2,3}$ 10 Hz, $J_{2,4}$ 1.7 Hz and $J_{3,4}$ 3.3 Hz.

As it is now well established, *cis* coupling in cyclic olefins shows a marked dependence on ring size, increasing with size [10, 11]. The value found for $J_{2,3}$ eliminates both cyclopentenic [9, 11] and *trans–trans* open chain olefinic [8] structures. The assignments of the signals for H_2 and H_3 are mainly based on the values of vinylic $J_{3,4}$ and allylic $J_{2,4}$ coupling constants. The angular dependence of such coupling in allylic systems has been rationalized [10, 13]. Although these relationships are only approximate, there is generally good agreement with experimental data. Moreover, it has been found [14, 15] that coupling of vinyl protons like H_b with proton such as H_a (i.e.—$CH_c = CH_b–CH_aX$) increase with the electronegativity of the substituent X, although theory predicts the opposite trend. On these

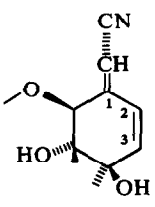
bases, it seems reasonable to relate the largest coupling constant to the vinyl group.

Spin decoupling experiments established coupling interactions between H_4 and H_7 ($J_{4,7}$ 1.0 Hz) and also with the proton located at 3.96 ppm ($J_{4,5}$ 6.2 Hz), H_5 is also coupled with H_6 at 4.86 ppm ($J_{5,6}$ 8.2 Hz). The signal centered at 4.86 ppm was then assigned to the allylic proton α to the *O*-glycosyl substituent, namely H_6. The low field value found for the allylic proton H_6 (4.86 ppm) compared to 4.30 ppm for H_4 seems to be in accordance with a stereoisomeric form in which the nitrile group is *syn* vs the glycosidic bond. Indeed, in such a configuration the anisotropic effect of the triple bond should deshield proton 6.

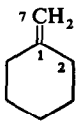
The PMR spectrum of the fully acetylated cyanoglucoside (**2**) requires some comments. First, there is no downfield shift of H_6 (4.75 ppm in **2** comp. 4.86 in **1**); however it should be noted an important deshielding effect for protons H_4 and H_5: 5.40 instead of 4.30 ppm for H_4 and 5.05 instead of 3.96 ppm for H_5. Proton H_6 gives a doublet of doublets whose smallest coupling constant corresponds to a long-range coupling with the olefinic proton H_7 ($J_{6,7}$ 1.5 Hz) as shown by double irradiation of H_7. The vicinal coupling constant with H_5 is $J_{5,6}$ 8.2 Hz. The methyl pattern of (**2**) shows six acetoxy groups, two of them belonging to the aglycone, four to glucose. The remaining signals in the spectrum of (**1**) confirm the presence of this sugar moiety. Furthermore the signal of the anomeric proton at 4.90 ppm is partially superimposed with the H_6 quartet; protons H_{6a} and H_{6b} form the AB part of an usually ABX system while $H_{5'}$ being the proton X. The close chemical shifts for protons 5′ and 2′ (unresolved multiplet at 3.52 ppm) is proved by the simultaneous formation of a quartet for protons H_6, and a sharp singlet for the anomeric proton by irradiation of the signal centered at 3.25 ppm. On the other hand $H_{3'}$ and $H_{4'}$ give a multiplet at about 3.44 ppm.

As often found in the PMR spectra of complex glucosides, the anomeric proton does not appear as a clear doublet but as a complex unresolved multiplet due to the non-equivalence of rotamers [16]. Despite the unavailability of $J_{1'2'}$, the β-anomeric configuration is supported by the $H_{1'}$, $H_{3'}$ and $H_{5'}$ shifts. In the α configuration the first proton would have been shielded and the two others deshielded by about 0.3–0.5 ppm. Finally, it is interesting to note a very unusual long-range coupling constant $J_{7,4}$ 1 Hz.

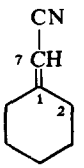
Catalytic hydrogenation of (**1**) gives the corresponding saturated glycoside (**3**) as an 1:2 mixture owing to the two possible configurations for the $-CH_2CN$ side chain. Both (**3**) and its acetate (**4**) show two $H_{1'}$ doublets ($J_{1'2'}$ 7 Hz) at 4.60 and 4.70 ppm for the former and 4.62 and 4.66 ppm for the latter. The stereochemistry of the three hydroxyl groups in the aglycone can be deduced from the values found for $J_{4,5}$ and $J_{5,6}$. Indeed, they are in a *trans–trans* relationship. Of course, the allylic



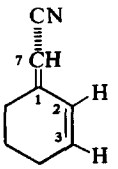
1



5



6



7

situation of both C_6 and C_4, the corresponding deformation of the six-membered ring and, in addition, the electron withdrawing effect of the oxygen atom might explain the smaller values of $J_{4,5}$ and $J_{5,6}$ than that expected for a perfect *trans* diaxial configuration.

Hydrogenation of the acetylated compound (**2**) provides a pure tetrahydro derivative (**4**) in one diastereoisomeric form with an axial oriented CH_2CN side chain. The H_6 proton is shifted to 3.8 ppm as a quartet with $J_{6,5}$ 8.1 Hz and $J_{6,1}$ 4.0 Hz and the anomeric proton shows a well resolved doublet $J_{1,2}$ 7.7 Hz at 4.62 ppm.

Further evidence in favour of structure (**1**) was obtained by ^{13}C NMR analysis (Table 1). This spectrum displays a strongly deshielded olefinic carbon at 155.9 ppm (C_1), tetrasubstituted as it appears from the undecoupled spectrum, and another strongly deshielded olefinic carbon at 97.4 ppm (C_7). Both chemical shifts are in good agreement with the partial structure depicted in (**7**).

Data given above for (**7**) agree reasonably well with those found for (**1**) except for the high value of J_{C_7-H} 176 Hz. The anomeric β-configuration of the glucose moiety is consistent with the chemical shift noted for C_1 102.7 ppm in (**1**) compared to 104.2 and 100.1 ppm respectively for β- and α-methylglucoside [18]. Moreover, the value $J_{C1'H}$ 162 Hz is in the range found for β-glucosides (i.e. 160 Hz) compared with the value (170 Hz) for the α-anomers [19].

Structure (**1**) is also consistent with the UV data, which correlate well with those for 1-cyanomethylenecyclohexane (**6**) (λ_{max} 217 nm) and 1-cyanomethylene-2-cyclohexene (**7**) (λ_{max} 258 nm). Comparing the last value with that for a corresponding diene, the contribution of the CN group appears to be about 30 nm.

Further evidence was gained from the MS of (**1**) and (**2**). The first shows besides the classical fragmentation of glucose the fragment ion peak *m/e* 149–150 ($C_6H_7NO_2$) corresponding to the aglycone. As usual for glucosides, M^+ at 329 is very weak. In the MS of the acetylated compound (**2**) there is the usual fragmentation of tetraacetylglucose, particularly the peak *m/e* 331 of the oxonium ion. Its presence excludes the possibility of a C-

Table 1. ^{13}C-NMR chemical shifts (δ ppm) and coupling constants (J Hz)*

Compound	(1)	(5)	(6)	(7)†
C_1	155.9	149.8	168.5	157.7
C_2	127.3(168)			127.6
C_3	136.6(163)			138.1
C_7	97.4(176)	106.5	92.0(172)	93.0(176.7)
CN	118.0		116.8	117.4

*In $CDCl_3$, downfield from internal TMS. †Carbons 1, 2 and 3 are split respectively 158, 128 and 139.7 ppm. Splitting results from the presence of two geometric isomers around the C_1-C_7 double bond.

glucosidic linkage [20]. Peaks at 223–224 *m/e* are assigned to the acetylated aglycone fragment.

Several attempts to isolate the aglycone chemically or enzymically were fruitless (see Experimental). Similar difficulties were encountered by Elliger *et al.* [28] working with the related structure 2-cyanomethylene-3-hydroxy-4,5-dimethoxy-cyclohexyl-β-D-glucoside from *Simmondsia californica.*

EXPERIMENTAL

Mp's are uncorrected. All NMR spectra were recorded in D_2O or in $CDCl_3$ (10%) with TMS as internal standard. MS were monitored at 70 eV using a direct inlet.

Extraction of plant material. Wild *L. purpureo-caeruleum* roots were harvested in the department of Gard (France), *L. officinale* was grown in Gif-sur-Yvette (France). Fresh roots were immediately extracted with boiling EtOH and the extract was conc. Residue was treated with lead acetate followed by mercuric acetate and finally by H_2S and by Et_2O extraction. After evaporation of solvent the oily residue was washed with warm H_2O–Me_2CO (9:1) several times. This soln was decolorized with charcoal and conc. Fine needles separated on cooling.

Isolation of lithospermoside (**1**). The product was purified by PC on Whatman no. 1 or 3 MM with *n*-BuOH–HOAc–H_2O (4:1:5, upper layer) the band at R_f 0.60 detected under short UV being eluted and rerun in *n*-BuOH–EtOH–H_2O (20:5:1) (BEW) or *i*-C_3H_7OH–EtOAc–H_2O (7:1:2) (AEW). Repeated cryst. from MeOH–H_2O (5:1) afforded pure (**1**): mp 278–279°, $[\alpha]_D^{20}$ − 156° (*c* 0.99, H_2O); λ_{max} 260 nm (ε, 21400); IR (KBr) 2220, 1630, 1606 cm^{-1}. Found: C, 51.10; H, 5.91; 0,38.95; N, 4.25 Calc. for $C_{14}H_{19}NO_8$ (329): C, 51.06; H, 5.82; O, 38.87; N, 4.25. Yields are 25 mg/kg root of *L. purpureo-caeruleum* and 98 mg/kg root of *L. officinale.*

Hydrolysis. Acidic hydrolysis of (**1**) (100 mg) was performed with 1.3 N H_2SO_4 (13 ml) (80°, 5 hr). After cooling, the mixture was extd. with Et_2O. The organic layer was dried and evaporated giving a yellow oily residue (30 mg) with a positive $FeCl_3$ and Barton's reagent tests. Preparative PC gave a strongly fluorescent band at R_f 0.80 (in BEW) and 0.82 (in AEW). Bands were eluted and product dissolved in $CHCl_3$ and added to an ethereal soln of excess CH_2N_2 and a few drops of MeOH: after 24 hr at 0°, extraction of the neutral products with Et_2O, filtration on a short Si gel column, evaporation and saponification with methanolic KOH gave 10 mg of an acid, repeatedly crystallized from Et_2O–hexane with a mp 87°. Found: C, 60.91; H, 6.11 Calc. for $C_{10}H_{12}O_4$: C, 61.2; H, 6.12 in good agreement with 2,3-dimethoxyphenylacetic acid (lit. [29] mp 85°). The aq. soln from the above hydrolysis was neutralized by $BaCO_3$, the salts separated by filtration and the liquid phase lyophilized leaving a residue which was dissolved in 95° EtOH: the crystalline material separated after 24 hr, was collected and identified as glucose by mp, mmp, optical rotation and osazone formation. Glucose % determination by the anthrone [26] and presulfonated resorcinol [27] methods gave 54.9% mole of (**1**). Glucose was isolated together with an untractable oily residue by treatment of (**1**) with emulsin for 3 days at 32°.

Lithospermoside hexa-acetate. Prepd. using Ac_2O–Py or Ac_2O–NaOAc and obtained as a clear syrup. Found: C, 53.3; H, 5.9; N, 2.4 Calc. for $C_{26}H_{31}NO_{14}$ (581): C, 53.7; H, 5.33; N, 2.4.

Tetrahydrolithospermoside (**3**) *hexa-acetate* (**4**). (**1**) (50 mg) in H_2O (30 ml) was hydrogenated over Pd/C (10%) at room temp. and atm. pres. The first mole of H_2 was taken up rapidly, the second more slowly. After filtration and lyophilisation a powdered hygroscopic residue was isolated (**3**) used without purification for NMR analysis. Acetylation of (**3**) with Ac_2O–NaOAc gave crystals from light petrol–Et_2O mp 140–142°. Found: C, 53.7; H, 5.28; N, 2.35 Calc. for $C_{26}H_{35}NO_{14}$ (585): C, 53.7; H, 5.33; N, 2.4. Hydrogenation of lithospermoside hexa-acetate (**2**) gave the same product but with a slightly different PMR spectrum (see theoret. part).

Cyclohexylidenacetonitrile (**6**). Prepared by condensation of cyclohexanone with cyanacetic acid as described by Cope *et al.* [23] or by Wittig reaction [24]. PMR: ppm 5.08 (*brd, s*) 2.33 (4H, *m*, H_2 and H_6) 1.6 (6H, broad *s*, H_3H_4 and H_5).

1-Cyanomethylene-2-cyclohexene (**7**). From treatment of (**6**) with NBS and dehydrohalogenation with $LiCO_3$ and dimethylformamide [25] (1 hr, 80°). Obtained in 50% yield as an oil. UV λ_{max} 258 nm (ε, 18200) PMR: ppm 6.66, 6.45, 6.30 (AB system) and 4.90 (*brd, s* H_7).

REFERENCES

1. Train, P., Henrichs, J. R. and Archer, W. A. (1941) *Medicinal Uses of Plants by Indian Tribes of Nevada. Contributions toward a Flora of Nevada.* Quoted by ref. 4.
2. Kemper, F. and Loeser, A. (1957) *Arzneimitt. Forsch.* **7**, 81; (1959) **9**, 368, 411 and (1961), **11**, 92.
3. Graham, R. C. B. and Noble, L. (1955) *Endocrinology* **56**, 239. Brenan, W. L. *et al.* (1960) *ibid.*, **67**, 583.
4. Kelley, Ch. J., Mahajan, J. R., Brooks, L. C., Neubert, L. A., Bren Eman, W. R. and Carmack, M. (1975) *J. Org. Chem.* **33**, 105.
5. Wagner, H. and Wittmann, D. (1975) *Tetrahedron Letters* 546.
6. Sosa, A., Sosa-Bourdouil C. (Mme) and Hardy, C. (1955) *C.R. Acad. Sci.* **240**, 1570.
7. Gorman, M., Bien, S. and Ginsberg, D. (1956) *Bull. Res. Council Israel* **5A**, 253.
8. Ettlinger, M. (1972) *J. Chem. Soc.* 572.
9. Coburn, R. and Long, L. (1966) *J. Org. Chem.* **31**, 4312.
10. Bothner-By, A. A. (1965) *Adv. Magnet. Res.* **1**, 149.
11. Smith, G. V. and Kriloff, H. (1965) *J. Am. Chem. Soc.* **85**, 2015.
12. Russel, G. B. and Reay, P. F. (1971) *Phytochemistry* **10**, 1373.
13. Karplus, M. (1960) *J. Chem. Phys.* **33**, 1842.
14. Bothner-By, A. A., Castellano, S. and Granier, H. (1965) *J. Am. Chem. Soc.* **87**, 2489.
15. Cohen, A. and Shaeffer, T. (1965) *Molec. Phys.* **10**, 209.
16. Mabry, T. J. (1970) in *Determination and Interpretation of NMR Spectra of Flavonoids* ch. VIII, p. 268. Springer, New York.
17. Garbisch, E. W. (1964) *J. Am. Chem. Soc.* **86**, 5561.
18. Grover, S. M. and Stothers, J. B. (1975) *Can. J. Chem.* **55**, 589.
19. Bock, K. and Pedersen, Chr. (1974) *J. Chem. Soc. (Perkin I)* 293.
20. Budzikiewicz, M., Djerassi, C. and Williams, D. H. (1964) in *Structure Elucidation of Natural Products by Mass Spectrometry* vol. II, p. 306. Holden-Day, San Francisco.
21. Conn, E. E. (1969) in *Perspective in Phytochemistry* (Harborne, J. B., ed.). Academic Press, London.
22. Carmack, M. personal communication.
23. Brande, A. and Wheeler, O. (1955) *J. Chem. Soc.* 322.
24. Bose, A. K. and Dahill, R. T. (1965) *J. Org. Chem.* **30**, 505.
25. House, H. O. and Bashe, R. N. (1965) *J. Org. Chem.* **30**, 2942.
26. Scott, T. A. and Melvin, E. H. (1953) *Analyt. Chem.* **25**, 1656; Zentner, H. (1965) *Analyst* **81**, 483.
27. Devor, A. W., Conger, C. and Gill, I. (1958) *Arch. Biochem. and Biophys.* **73**, 20.
28. Ellinger, C. A., Waiss jr, A. C. and Ludin, R. E. (1972) *J. Chem. Soc. (Perkin I)* 2209; *ibid.*, (1974) *Phytochemistry* **13**, 2319.
29. Spath and Mosettig, E. (1923) *Annalen* **433**, 140, 145.