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Degraded cyanogenic glucosides from *Sambucus nigra*

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Abstract

Three unusual cyanohydrins, which might be formed by oxidative cleavage of cyanogenins, have been isolated from *Sambucus nigra*. The structures of the compounds have been defined on the basis of spectroscopic features and by chemical degradation. Unlike cyanogenins the title compounds do not have phytotoxic effects and might be involved in the detoxification process of the plant.

Keywords: Sambucus nigra; natural cyanohydrins; acetals.

In a study of the detrimental effects on crops of *Sambucus nigra*, a weed found in Southern Italy, we have recently reported[1] the presence of cyanogenic glycosides in the aqueous fraction of the methanolic extract of the plant, sambunigrin (**1**) and prunasin (**2**) being the most abundant.

In pursuing the chemical investigation of this plant we have isolated, from the EtOAc fraction of the same extract, three cyanohydrins **3**–**5**, which could be involved in the detoxification process of the plant.

Compound **3**[2] ($[\alpha]_D$ +15) has a molecular peak at m/z 353 in the EI mass spectrum and its elemental analysis agrees with the molecular formula $C_{16}H_{19}NO_8$. The IR spectrum shows hydroxyl stretches at 3619 and 3508 cm^{-1} and a carbonyl stretch at 1747 cm^{-1}. According to the molecular formula, in the ^{13}C NMR spectrum there are 14 carbon signals present, two of them integrating for two carbons in an inverse-gated experiment. ^1H and ^{13}C NMR spectral data obtained by COSY, HMQC and HMBC (Table 1) in $CDCl_3$ allow definition of a rough structure. Beside the heterocorrelations with the nitrile and the aromatic carbons, the H-2 proton at δ 5.90 gives a cross peak with the acetal carbon at δ 100.1. To this carbon the H-2′ proton at δ 4.60 and the H-5′ proton at δ 4.69 are also correlated. The first proton gives a further cross peak with the C-3′ carbon while the latter has correlations with both the C-4′ and the C-6′ carbons. Finally, the acetal proton at δ 5.32 has connectivities with the C-2, C-2′, C-3′ and C-5′ carbons.

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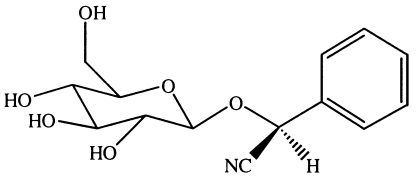

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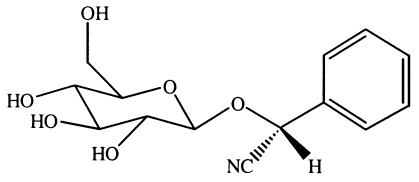

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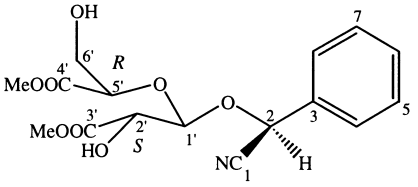

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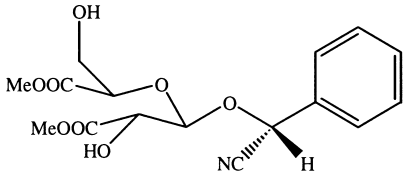

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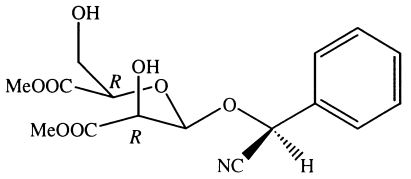

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Table 1
^{1}H and ^{13}C NMR spectral data of compound **3**

Position	^{13}C	DEPT	^{1}H	*J* (Hz)	HMBC
1	117.3	C			
2	66.9	CH	5,90 s		C1, C3, C4, C8 C1'
3	133.2	C			
4	128.1	CH	7.43 m		
5	127.3	CH	7.43 m		
6	130.0	CH	7.43 m		
7	127.3	CH	7.43 m		
8	129.1	CH	7.43 m		
1'	100.1	CH	5.32 d	2.6	C2, C2', C3', C5'
2'	72.9	CH	4.60 d	2.6	C1', C3'
3'	170.8	C			
4'	170.5	C			
5'	77.3	CH	4.69 dd	3.2 and 5.6	C1', C4', C6'
6'	63.3	CH_2	3.93 dd 4.08 dd	5.6 and 10.4 3.2 and 10.4	C4', C5' C4', C5'
OMe	52.8	CH_3	3.68 s		C4'
OMe	53.2	CH_3	3.84 s		C3'

When the ^{1}H NMR spectrum of **3** is registered in CD_3OD the H-2 proton signal is found at δ 6.02 and this value is consistent with the presence of the (*S*) mandelonitrile moiety as found in sambunigrin (**1**).

Cyanide **3** has been converted into the two diastereomeric MTPA diesters: the diester derived by treatment with (*S*)-(+)-MTPA-Cl shows in the ^{1}H NMR spectrum in $CDCl_3$ the H-1′ doublet at δ 5.49 while the derivative from (*R*)-(–)-MTPA-Cl shows the same proton at δ 5.46. According to Mosher,[3] these data indicate the *S*-configuration at C-2′. TFA hydrolysis of **3** followed by $NaBH_4$ reduction gives the racemic methyl ester of glyceric acid. In this way the *R*-configuration is determined at C-5′. These results support the possibility that **3** might derive from sambunigrin (**1**) by oxidative cleavage of the C-3′–C-4′ bond of the glucose moiety and, following such a hypothesis, the *R*-configuration at C-1′ has been assumed.

Compound **4**[4] ($[\alpha]_D$ –21) isolated in small amounts along with **3**, has an identical EI mass spectrum, IR and ^{13}C NMR spectra while showing the H-2 and H-1′ protons upfield shifted at δ 5.72 and 5.05, respectively, in the ^{1}H NMR spectrum. Furthermore, the H-2 proton resonates at δ 5.90 in the spectrum taken in CD_3OD. The small differences in **3** and **4** have already been observed in the comparison to the NMR data of sambunigrin (**1**) and prunasin (**2**) and it can be postulated that **4** might derive from prunasin by oxidative cleavage of the C-3′–C-4′ bond.

Also compound **5**[5] ($[\alpha]_D$–3) has a molecular formula of $C_{16}H_{19}NO_8$ according to the EI mass spectrum and ^{13}C NMR data. While the ^{13}C NMR spectrum is rather similar to that of **3**, the ^{1}H NMR spectrum shows the H-2 proton at δ 6.02, the H-1′ and H-2′ doublets at δ 5.25 and 4.38, respectively, the H-5′ double doublet at δ 4.52 and the H-6′ double doublets at δ 3.89 and 4.06. The chemical shift of H-2 in CD_3OD accords with the presence of *S*-mandelonitrile as the aglycone.

The configurations at C-2′ and C-5′ were defined as has already been reported for **3**. Compound **5** was treated with *S*- and *R*-MTPA-Cl. In the ^{1}H NMR spectrum of the diester from *S*-(+)-MTPA-Cl the H-1′ proton resonates at δ 5.62 whilst that of the *R*-(–)-MTPA derivative is at δ 5.66, consistent with *R*-configuration at C-2′. Trifluoroacetic acid hydrolysis and $NaBH_4$ reduction affords the methyl ester of *R* glyceric acid proving the *R*-configuration assigned to both the C-2′ and C-5′ carbons. Differently from glucosides sambunigrin and prunasin, (*S*)-*O*-β-D-mannopyranosylmandelonitrile is not a metabolite of *S. nigra* so that it is likely that **5** is formed from **3** by epimerization at C-2′.

Compounds **3**–**5**, as well as sambunigrin (**1**) and prunasin (**2**), have been assayed for their phytotoxicity using the germination inhibition of *Raphanus sativum* L. and *Lactuca sativa* L.[6] as a test. The assays show that sambunigrin and prunasin cause strong inhibition while at the same concentrations compounds **3**–**5** have a slight stimulant effect.

Studies[7] on the species *Sorghum bicolor* and *Linum usitatissimum* have shown that the phytotoxicity of the cyanogenins is due to the release of HCN by action of glycosidase and hydroxynitrile lyase. Autotoxic effects are avoided by separating substrates on subcellular levels from enzymes. When a sample of **1** is added to an aqueous extract of *S. nigra,* the formation of benzaldehyde proves that the plant has the enzymes able to release HCN.[8] According to the previous toxicity assays, when **3** is added to the same extract, no trace of benzaldehyde is detected. Such data suggest that **3**–**5** are not substrates for the enzymes and it is possible that they could be involved in a catabolic pathway that the plant uses for its detoxification.

References

1. DellaGreca, M.; Fiorentino, A.; Monaco, P.; Previtera, L.; Simonet, A. M. *Nat. Prod. Letters* **2000**, *14*, 175–182.

2. Compound **3**: $[\alpha]_D$ +15 (*c* 0.6, $CHCl_3$); EIMS 353 $[M]^+$, 237 $[M–C_8H_6N]^+$, 221 $[M–C_8H_6NO]^+$, 118 $[C_4H_6O_4]^+$; UV λ_{max} (EtOH) 221 and 278 (ε 3150 and 2170); IR ν_{max} ($CHCl_3$) 3619, 3508, 1747 cm^{-1}; anal. calcd for $C_{16}H_{19}NO_8$: C, 54.39; N, 3.96; H, 5.42%. Found: C, 54.31; N, 3.99; H, 5.30%.
3. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1972**, *95*, 512–519.
4. Compound **4**: $[\alpha]_D$ –21 (*c* 0.3, $CHCl_3$); EIMS 353 $[M]^+$, 237 $[M–C_8H_6N]^+$, 221 $[M–C_8H_6NO]^+$, 118 $[C_4H_6O_4]^+$; UV λ_{max} (EtOH) 225 and 276 (ε 3160 and 2190); IR ν_{max} ($CHCl_3$) 3621, 3510, 1749 cm^{-1}; ^1H NMR δ 7.43 (5H, m, H-4–H-8), 5.72 (1H, s, H-2), 5.05 (1H, d, J = 2.6 Hz, H-1′), 4.46 (1H, d, J = 2.6 Hz, H-2′), 4.57 (1H, dd, J = 3.3 and 5.6 Hz, H-5′), 4.08 (1H, dd, J = 3.3 and 10.4 Hz, H-6′), 3.81 (1H, dd, J = 5.6 and 10.4 Hz, H-6′), 3.65 (3H, s, OMe), 3.84 (3H, s, OMe); anal. calcd for $C_{16}H_{19}NO_8$: C, 54.39; N, 3.96; H, 5.42%. Found: C, 54.33; N, 4.11; H, 5.32%.
5. Compound **5**: $[\alpha]_D$ –3 (*c* 0.5, $CHCl_3$); EIMS 353 $[M]^+$, 237 $[M–C_8H_6N]^+$, 221 $[M–C_8H_6NO]^+$, 118 $[C_4H_6O_4]^+$; UV λ_{max} (EtOH) 224 and 279 (ε 3130 and 2130); IR ν_{max} ($CHCl_3$) 3618, 3509, 1747 cm^{-1}; ^1H NMR δ 7.51 (2H, m, H-4 and H-8), 7.43 (3H, m, H-5–H-7), 6.02 (1H, s, H-2), 5.25 (1H, d, J = 4.0 Hz, H-1′), 4.38 (1H, d, J = 4.0 Hz, H-2′), 4.52 (1H, dd, J = 3.2 and 6.7 Hz, H-5′), 4.09 (1H, dd, J = 3.2 and 10.4 Hz, H-6′), 3.93 (1H, dd, J = 6.7 and 10.4 Hz, H-6′), 3.68 (3H, s, OMe), 3.84 (3H, s, OMe); anal. calcd for $C_{16}H_{19}NO_8$: C, 54.39; N, 3.96; H, 5.42%. Found: C, 54.27; N, 4.06; H, 5.37%.
6. Macias, F. A.; Simonet, A. M.; Galindo, J. C. G.; Pacheco, P. C.; Sanchez, J. A. *Phytochemistry* **1998** , *49*, 709–717.
7. Wajant, H.; Riedel, D.; Benz, S.; Mundry, K. W. *Plant Sci.* **1994**, *103*, 145–154.
8. A cold homogenate of fresh leaves (100 g) from *S. nigra*, collected in the Spring, in phosphate buffer (500 mL, 5 mM, pH 7.2) containing NaCl (70 mmol), was filtered on Miracloth paper and centrifuged at 4°C for 90 min (4000 rpm). The supernatant liquor was precipitated with 40% $(NH_4)_2SO_4$ and then with 100% $(NH_4)_2SO_4$. The proteins precipitated were dissolved in distilled water and dialyzed against water to eliminate salts. To the soluble proteinaceous fraction (50 μL), obtained by centrifugation of the material, sambunigrin (100 μg), acetate buffer (100 μL, 0.5 M) and water (750 μL) was added and the reaction mixture was kept at 37°C for 24 h. The presence of benzaldehyde was detected by UV and TLC comparison with an authentic sample.