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Short Communications

Cyanogenic Glucosides in *Sambucus nigra* L.

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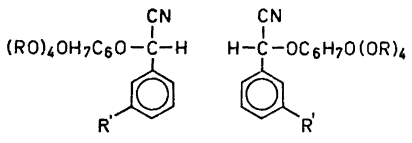
The cyanogenic glucoside sambunigrin (*1*) has been repeatedly identified as a constituent of common elder (*Sambucus nigra* L., Caprifoliaceae).[1] We report the additional occurrence in certain elder collections of prunasin (*2*) and the *m*-hydroxysubstituted glucosides zierin [2] and holocalin [3] (*3* and *4*).

Frozen leaf material was extracted with ethanol and worked up to give a mixture of cyanogenic glucosides which could be separated by chromatography into pairs of epimers (see Experimental). The mixture of sambunigrin *1* and prunasin *2*, was readily identified by PMR-analysis, the epimers differing conspicuously in the chemical shifts of the benzylic protons: δ 6.07 and 5.88 ppm, respectively. Acetylation, followed by chromatographic separation, gave the corresponding acetates *5* and *6*, possessing properties identical with those previously reported (see Table 1) including the characteristic differences in the PMR-spectra (*cf.* Ref. 4).



1 : R = R' = H 2 : R = R' = H
3 : R = H, R' = OH 4 : R = H, R' = OH
5 : R = Ac, R' = H 6 : R = Ac, R' = H
7 : R = Ac, R' = OAc 8 : R = Ac, R' = OAc

Analogously, PMR analysis revealed the identity of the second fraction as a mixture of *3* and *4*, again exhibiting benzylic proton signals at 6.07 and 5.88 ppm, respectively. Acetylation, followed by chromatographic separation of the mixture, afforded the pentaacetates *7* and *8*. Their PMR-spectra exhibited differences similar to those observed above for the pair *5* and *6*, and for the acetates *9* and *10*, derived from the epimeric pair of *p*-hydroxy-substituted isomers (dhurrin-taxiphyllin) [4] (see Table 1). Additional to the characteristic differences in the chemical shift of the benzylic protons, the acetoxy patterns of the epimers display differences which are diagnostically useful.

On this basis, *7* is considered a derivative of a member of the (*S*)-cyanohydrin series, thus far represented by sambunigrin and dhurrin. Consequently, *8* derives from an (*R*)-cyanohydrin, configurationally related to prunasin and taxiphyllin. The pairwise differences in melting points and specific rotations of the acetates are consistent with this assignment (*cf.* Table 1). Acetylation of holocalin, kindly provided by Dr. R. Gmelin, afforded a pentaacetate, indistinguishable (m.p., m.m.p., PMR-spectrum) from *8*. Hence, holocalin *4* belongs to the (*R*)-series, a conclusion opposite to that arrived at by Gmelin *et al.*[3] (Parallel to our work, Nahrstedt [5] has reached the same conclusion, based on the GLC-behaviour of TMS-ethers of the epimers.)

A number of collections of very young shoots from different localities have been examined. Out of six samples, collected on widely separated localities on the island of Sjælland, four contained *1* as the sole detectable cyanogenic glucoside, demonstrating that no isomerization had taken place during the standard work-up. Only two collections contained the *m*-hydroxy-substituted glucoside(s) as apparent from PMR-data. Further work to clarify the distributional pattern is in progress.

Table 1.

	m.p. °C	$[\alpha]_D^{21}$ (°; in $CHCl_3$)	PMR-data H_α	H_1'	H_6'	H_6'	H_5'	OAc-pattern	Glucoside content (% of fresh weight)
5	125–126[a]	−55[a]	5.71	5.00	4.32	4.25	3.83	1:1:1:1	0.044
6	137–138[b]	−17[b]	5.54	4.57	4.27	4.17	3.69	1:3	0.018
7	117.5–118.5[d]	−50[c]	5.73	4.98	4.30	4.23	3.81	1:1:1:1:1	0.016
8	129–130	−12	5.56	4.59	4.28	4.15	3.69	1:1:3	0.022
9[e]	132–133	−50[f]	5.69	5.02	4.28		3.84	1:1:1:1:1	
10[e]	144–145	−22[f]	5.54	4.62	4.20		6.68	1:1:3	

[a] Reported:[6] m.p. 125–126°; $[\alpha]_D^{21}$ −54° (EtOAc). [b] Reported:[6] m.p. 139–140°; $[\alpha]_D^{21}$ −24° (EtOAc). [c] In EtOH: −46°. [d] Reported:[2] m.p. 115–118°; no rotation data listed. [e] Quoted from Ref. 4. [f] Measured in EtOH.

Experimental. Melting points are corrected and determined in capillary tubes in a heated bath. PMR-spectra are recorded on a Varian HA-100 instrument, with TMS as an internal standard. Preparative TLC was performed on 20 × 40 cm plates coated with 1 mm layers of silica gel PF_{254} (Merck); detection by UV-light. Analyses were performed by Dr. A. Bernhardt.

Isolation of glucosides. Frozen plant material (210 g, collected in June 1972 and stored for 9 months in polyethylene bags at −25°) was homogenised with ethanol and worked up as previously described.[7] The mixture of cyanogenic glucosides (245 mg) was separated from the other glycosides (0.74 g) by preparative TLC, with EtOAc–Bz–EtOH (4:1:1) as an eluent. A second fractionation, this time with $CHCl_3$–MeOH (4:1) as the eluent, separated the mixture into two fractions, a faster moving, *A*, (*1* and *2*, 134 mg), and a slower moving, *B* (*3* and *4*, 83 mg). Fraction *A* was acetylated (Ac_2O/Py), and the resulting mixture of acetates was subjected to preparative TLC (ether–pentane; 9:1) to give, as the faster moving fraction, *sambunigrin tetraacetate* (*5*, 99 mg) and as the slower moving fraction, *prunasin tetraacetate* (*6*, 42 mg). Data are presented in Table 1. Fraction *B* was treated similarly to give *zierin tetraacetate* (*7*, 47 mg) (Found: C 55.35; H 5.34; N 2.80. Calc. for $C_{24}H_{27}NO_{12}$: C 55.28; H 5.22; N 2.69) and *holocalin tetraacetate* (*8*, 64 mg) (Found: C 55.14; H 5.28; N 2.80). Further data are presented in Table 1.

Vouchers (IOK 30/72: IOK 3/73; IOK 5/73; IOK 8/73 – IOK 12/73) have been deposited at the Botanical Museum of the University of Copenhagen, Denmark.

Added in proof. Collections made in September all contain holocalin, exclusively, or in admixture with zierin. The contents ofsambunigrin were low.

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