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PRUNASIN, THE CYANOGENIC GLYCOSIDE IN *AMELANCHIER ALNIFOLIA*

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The proportion of Saskatoon serviceberry (*Amelanchier alnifolia* Nutt.) in the diet of free-ranging mule deer (*Odocoileus hemionus hemionus*) varies from low to moderate [1, 2]. Nevertheless, field observations on the plant in the Interior of British Columbia indicate a high degree of browsing [3]. Penned, mature mule deer, however, ingesting 1 kg fr. wt per day *A. alnifolia* went off their feed and developed shortness of breath and lack of muscle control after 7 days on the diet in early March of 1977. The experimental animals expired within 24 hr of the first indication of any sickness, despite an intense effort to save them [4]. *Amelanchier* species are not found in lists of toxic plants [5, 6] and, in fact, *A. alnifolia* is a recommended species for habitat improvement plantings in game management [7, 8].

Samples of the above feed gave a positive HCN test with picrate paper. Cyanogenesis has been reported in eight species of *Amelanchier* [9, 10] including seeds of *A. alnifolia*, but the cyanogenic compound(s) have not been characterized. This present work describes the isolation and identification of prunasin, the cyanogenic glycoside in Saskatoon serviceberry.

The isolate, obtained as 0.15% fr. wt *A. alnifolia* twigs and buds, yielded HCN, glucose and benzaldehyde following treatment with almond emulsin. HCN was identified by the picrate paper test, glucose by the *p*-anisidine phthalate reagent following PC, and benzaldehyde by GLC. The isolate co-chromatographed with an authentic sample of prunasin (courtesy Dr Eric E. Conn) in five solvents. The TMS derivative of our isolate co-chromatographed with the TMS derivative of the reference sample of prunasin using the GLC conditions described by Butterfield *et al.* [11]. These GLC conditions resolved TMS–prunasin from TMS–sambunigrin. Sambunigrin, the diastereoisomer of prunasin, was readily generated from prunasin by treatment with 0.01 N NH_4OH for 1 hr at room temp. [12, 13]. The TLC purified material, twice recrystallized from EtOAc, melted at 144–148° (lit. 148–151° [14] and 147–148° [15]). Dissolved in water the isolate had $[\alpha]_D^{25}$ −24.0, *c*, 3.07 (lit. −30.1 [14] and −27.2 [15]). The ^{1}H-PMR spectrum of the D_2O exchanged product was in complete agreement with the assigned structure, giving a 5H multiplet δ 7.57 (aromatic), a 1H singlet δ 5.89 (cyanohydrin), 1H doublet δ 4.59, J = 6 Hz (anomeric), and the remaining 6H of the hexose moiety.

EXPERIMENTAL

Amelanchier alnifolia (100 g fr. wt, twigs and buds) was collected on 4 April, 1977, near Kamloops, B.C., and homogenized with 500 ml hot EtOH. The filtrate was concd, fractionated on activated coconut charcoal [16] and the fraction containing prunasin was eluted with 2 l. 50% C_6H_6 in EtOH. This fraction was concd to dryness, redissolved in 15 ml H_2O, applied to a polyamide-CC_6 (Macheray, Nagel and Co.) column (15 × 3 cm) equilibrated in H_2O and the prunasin fraction was eluted with 200 ml H_2O. This eluate was concd, applied to 10 Avicel-Si gel 7 (Baker No. 3406) TLC plates (1:1, 20 × 40 cm, 1 mm thick) and chromatographed in EtOAc. The prunasin band (located initially with the picrate paper sandwich test and subsequently by UV absorbance) was eluted with EtOH, concd and crystallized twice in EtOAc.

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