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SHORT REPORTS

CYANOGENESIS IN *ACACIA SUTHERLANDII*

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Key Word Index—*Acacia sutherlandii*; Leguminosae; cyanogenic glycosides; isolation; sutherlandin; proacacipetalin.

Abstract—Two cyano-glucosides have been isolated from leaves of *Acacia sutherlandii*. One is the previously described cyanogenic glucoside proacacipetalin and the other is the novel, non-cyanogenic, glycoside 1-cyano-2-β-D-glucopyranosyloxymethyl-(*Z*)-prop-1-en-3-ol which has been given the trivial name sutherlandin.

INTRODUCTION

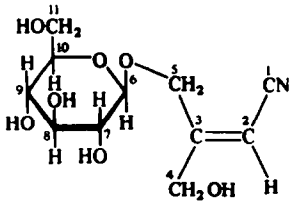
We have identified two nitrile-containing glycosides in an Australian legume, *Acacia sutherlandii*. One is the known cyanogenic glycoside proacacipetalin which was identified by ^{1}H NMR. The second is a novel non-cyanogenic cyano-glucoside which we have named sutherlandin. *Acacia sutherlandii* belongs to the subgenus *Acacia*. It is endemic to Australia and is found only in Queensland and the Northern Territory. This species is a tree which grows to about 7 m and has rough, corky bark and drooping foliage. The placement of this species in the subgenus *Acacia* is reinforced by the presence of glycosides derived (or may be presumed to be derived) from the aliphatic amino acid L-leucine [1, 2].

RESULTS AND DISCUSSION

Sutherlandin (1), $C_{11}H_{17}NO_7$, was isolated by prep. HPLC as a white powder. The M_r, 275.0927, and molecular formula were determined using high resolution desorption chemical ionization mass spectrometry (DCI-MS). The ^{1}H NMR spectrum of 1 in D_2O showed the presence of a glycosidic moiety whose identity was confirmed as glucose by the glucose oxidase method. The coupling constant of the anomeric proton (δ4.50, J = 8 Hz, H-6) indicated that the glucose was attached to the remainder of the molecule via a β linkage [3]. Three sets of protons were seen in the ^{1}H NMR spectrum in addition to those assigned to glucose. There was a broad singlet, δ5.83, a doublet of doublets, δ4.63, and a doublet, δ4.39. The decoupled ^{13}C NMR spectrum of 1 contained 11 peaks, 5 of which were assigned to the glucose moiety with the 6th glucose carbon resonating at either δ60.8 or δ60.5 [4]. Of the remaining 5 carbons, one, δ116.4, was assigned to the nitrile group (C-1). Two carbons, δ66.3 and δ60.8 (or δ60.5) were triplets in the coupled spectrum and were tentatively identified as methylenes adjacent to oxygen. The two remaining carbons, δ164.8 and δ93.9, had uncertain identities. Given the molecular formula and NMR data, only one carbon skeleton was possible (1). The two unassigned carbons in the ^{13}C NMR spectrum were assigned to the vinylic carbons, δ164.8 (C-3) and δ93.9 (C-2). The atypical chemical shifts observed for these carbons were due to the conjugation effect of the adjacent cyano group [5].

The stereochemistry about the double bond was determined as (*Z*) by NOE experiments. When the signal due to the C-4 protons was irradiated, the vinylic proton (H-2) signal was enhanced, and, when the signal due to the C-5 protons was irradiated, no NOE effect was seen on the vinylic proton. This showed that the vinylic proton was closer in space to the C-4 protons than to the C-5 protons and confirmed the stereochemistry about the double bond as (*Z*). An NOE effect was also seen on the anomeric proton when the C-5 proton signal was irradiated which proved that the glucose was attached to C-5, not C-4. Therefore, the structure assigned to sutherlandin is 1-cyano-2-β-D-glucopyranosyloxymethyl-(*Z*)-prop-1-en-3-ol (1).

Sutherlandin has the same carbon skeleton as a compound which was isolated from the New Guinean bug *Leptocoris isolata* [6]. In the reported compound, the stereochemistry about the double bond was not defined and the ^{1}H NMR data presented differs from that obtained for sutherlandin. The chemical shifts assigned to the allylic alcohol protons in the reported



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compound are reversed from sutherlandin and, based on the NOE experiments done on sutherlandin, they are probably incorrect. Even taking this into account, there are significant differences in the ^{1}H NMR spectra (CD_3OD) of the two compounds; the reported compound is probably the (*E*) isomer of 1-cyano-2-β-D-glycopyranosyloxymethylprop-1-en-3-ol.

Sutherlandin contains the same glycolic fragment as the nitrile containing natural product known as cyanolipid II [5, 7]. In cyanolipid II, both of the allylic hydroxyl groups are esterified with fatty acids whereas in sutherlandin one of the allylic hydroxyl groups is glucosylated and the other is a free hydroxyl group.

EXPERIMENTAL

Plant material. Leaves and stems of *Acacia sutherlandii* were collected in Queensland, Australia, in August, 1984 (voucher: Norman Hall H84/27). A second batch of plant material was subsequently collected 20 km north of Longreach in Queensland on 9/28/85 (voucher: Pedley 5319). Voucher specimens were deposited in the Queensland Herbarium, Meiers Road, Indooroopilly, Queensland 4068, Australia.

Extraction and isolation. Leaves (174 g dry wt) were ground in boiling 95% EtOH in a Waring blender. The filtered soln was concd under vacuum and subsequently extracted with MeOH–$CHCl_3$–H_2O (12:5:3). Additional H_2O and $CHCl_3$ were added as necessary to separate the layers. The aq. layer was treated with 10% PbOAc and then H_2S. Open CC was performed first on polyamide (MN), solvent H_2O, and subsequently on cellulose, solvent MeCOEt–Me_2CO–H_2O (15:5:3). Final purification was via HPLC with *A* monitored at 200 nm. The extract was run on a Whatman C-18 ODS-3 column (9.4 mm × 50 cm) with a solvent of CH_3CN–H_2O (5:95) at a flow rate of 4 ml/min. Final purification was achieved on an Astec cyclobond I (10 mm × 50 cm) column using MeOH–H_2O (5:95) at a flow rate of 4 ml/min. Fractions containing **1** were collected, frozen and lyophilized to yield 16 mg. The compound was detected during purification using Feigl–Anger cyanide sensitive paper after hydrolysis of the glycoside using almond emulsin [8]. Compound **1** (found: *M*, 275.0942. $C_{11}H_{17}NO_7$ requires: 275.0927). ^{1}H NMR (500 MHz, D_2O): δ3.32–3.52 (4H, *m*, H-7, H-8, H-9 and H-10), 3.77 (1H, *dd*, $J_{11\alpha,11\beta}$ = 12 Hz, $J_{11\alpha,10}$ = 5 Hz, H-11α), 3.95 (1H, *dd*, $J_{11\beta,11\alpha}$ = 12 Hz, $J_{11\beta,10}$ = 2 Hz, H-11β), 4.39 (2H, *s*, H-4), 4.5 (1H, *d*, *J* = 8 Hz, H-6), 4.61 (1H, *d*, *J* = 14 Hz, H-5α), 4.65 (1H, *d*, *J* = 14 Hz, H-5β), 5.83 (1H, *s*, H-2). ^{1}H NMR (500 MHz, MeOH-d_4): δ3.32–3.52 (4H, *m*, H-7, H-8, H-9 and H-10), 3.77 (1H, *dd*, $J_{11\alpha,11\beta}$ = 12 Hz, $J_{11\alpha,10}$ = 5 Hz, H-11α), 3.95 (1H, *dd*, $J_{11\beta,11\alpha}$ = 12 Hz, $J_{11\beta,10}$ = 2 Hz, H-11_b), 4.32 (1H, *d*, *J* = 8 Hz, H-6), 4.39 (2H, *s*, H-4), 4.56 (1H, *d*, *J* = 14 Hz, H-5α), 4.66 (1H, *d*, *J* = 14 Hz, H-5β), 5.83 (1H, *s*, H-2). ^{13}C NMR (90.5 MHz, DMSO-d_6): δ60.5 (*t*, C-4 or C-11), 60.8 (*t*, C-4 or C-11), 66.3 (*t*, C-5), 69.5 (*d*, C-7), 72.9 (*d*, C-9), 76.2 (*d*, C-8), 76.7 (*d*, C-10), 93.9 (*d*, C-2), 102.4 (*d*, C-6), 116.4 (*s*, C-1), 164.8 (*s*, C-3).

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