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BUCHANANINE, A NOVEL PYRIDINE ALKALOID FROM *CRYPTOLEPIS BUCHANANI*

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Key Word Index—*Cryptolepis buchanani*; Asclepiadaceae; nicotinoyl glucopyranoside; buchananine.

Abstract—A new alkaloid, buchananine, was isolated from the stems of *Cryptolepis buchanani*. Its structure was determined as 6-*O*-nicotinoyl-α-D-glucopyranose by chemical and spectroscopic investigations.

INTRODUCTION

Cryptolepis buchanani (Asclepiadaceae) is a large glabrous, twining shrub with terete branches [1]. It grows more or less throughout the hotter parts of India [2]. The plant is used in a preparation given to children to cure them of rickets [3]. Decoctions of its stems are used by some rural people as a cure for paralysis [4].

There are about 28 *Cryptolepis* species on which practically no phytochemical investigation has been carried out. There are reports of preliminary phytochemical investigation of only two African species [5–7].

The medicinal property attributed to this plant and lack of phytochemical information prompted the present investigation and has resulted in the isolation of a new alkaloid, designated as buchananine.

RESULTS AND DISCUSSION

Dried and milled stems were defatted with petrol and then extracted with ethanol. The ethanol extract was concentrated under reduced pressure and the viscous residue was triturated successively with chloroform, acetone and 50% aqueous methanol. The aq. methanolic fraction on concentration gave an amorphous material which on crystallization from aq. methanol yielded buchananine (**1**), mp 137–38°.

Buchananine (**1**), $C_{12}H_{15}NO_7$, $[\alpha]_D^{20}$ + 38.4 (*c*, 3.08 in water); showed UV λ_{max}^{EtOH} 221, 258 (sh), 262 and 271 (sh) nm (log ε, 3.93, 3.43, 3.45 and 3.37, respectively) very similar to that of rostratine [8], an alkaloid which is an ester of nicotinic acid. The IR spectrum exhibited peaks for bonded OH (broad band at 3300 cm^{-1}), ester carbonyl (1730 cm^{-1}) and aromatic double bond (1600 cm^{-1}).

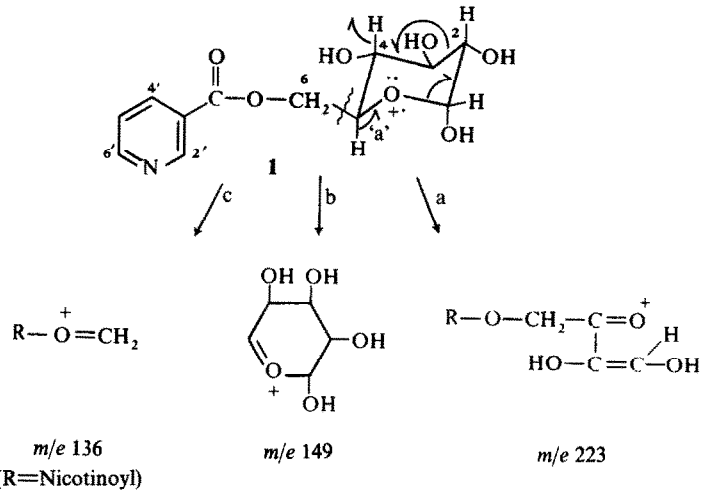
Hydrolysis of buchananine with N HCl in aq. methanol gave glucose and nicotinic acid. Glucose was isolated as its pentaacetate by acetylation of the vacuum dried hydrolysis product. The mother liquor, after removal of the acetate, was concentrated to afford nicotinic acid. Identity of the hydrolysis products was confirmed on the basis of UV, IR, NMR, MS, and comparison with authentic samples.

The PMR spectrum of the alkaloid, taken in D_2O, showed four aromatic protons. A 1H triplet at δ 8.18 (J = 7 Hz); a 2H multiplet at δ 9.0–9.2; and a 1H singlet, broad with fine splitting, at δ 9.33 were assignable to the C-5′, C-4′ and C-6′, and C-2′ protons, respectively of a 3-substituted pyridine ring [9]. The non-aromatic part of the PMR spectrum integrated for seven protons of the glucose moiety with a 3H singlet, broad with fine splitting, at δ 3.85 (C-5 and C-6), a 3H complex multiplet spread over the region δ 3.4–3.66 (C-2, C-3 and C-4) and a 1H doublet at δ 5.32 ($J_{1,2}$ = 3.5 Hz, C-1). The J value [10] of the anomeric proton indicated that the glucose moiety was in the α-form. From the integration of the aromatic and aliphatic protons, it was evident that the alkaloid was composed of nicotinic acid and glucose in equimolecular proportions. The C-6 hydrogens of the glucose moiety in buchananine resonated at a relatively higher field than generally expected for a CH_2 group attached to an ester function. This may be due to shielding [11] by the interatomic diamagnetic effect of the pyridine ring.

The above information indicated that nicotinic acid was esterified with α-D-glucose in buchananine. Since the alkaloid readily reduced Fehling's and Benedict's solution the C-1 hydroxyl of glucose was free. The spot of the alkaloid on TLC turned pink when sprayed with an alcoholic solution of 2,4-dinitrophenylhydrazine followed by 10% KOH solution indicating its aldehyde [12] character.

Periodic acid oxidation suggested that—CH_2OH [13] was involved in the ester linkage. When the alkaloid was treated with periodic acid in bicarbonate buffer [14] no formaldehyde-dimedone was obtained. Bell [15] has indicated that HIO_4 oxidation with production of formaldehyde affords a quantitative test for the presence of a free primary OH group. The alkaloid did not give trityl ether under the conditions [16] used for reaction at the primary alcoholic group. This provided additional evidence to show that the —CH_2OH in the glucose moiety was not free.

Buchananine did not give any molecular ion peak in its MS. Peaks at *m/e* 123 and 124 in the MS of the alkaloid were indicative of an esterified nicotinoyl group. The base peak at *m/e* 123 was probably formed by hydrogen transfer [17] often observed with esters of aliphatic alcohols. Other prominent peaks at *m/e* 124 ($C_6H_6NO_2$, protonated nicotinic acid), 106 (nicotinoyl), 105 (nicotinoyl-H), 78 (pyridyl) which are also present in the MS of nicotinic acid itself, could have arisen either directly from the molecular ion or from the *m/e* 123 fragment.



The genesis of the peaks at *m/e* 223, 149 and 136 in the MS of the alkaloid can be rationalized on the basis of structure **1** for buchananine.

EXPERIMENTAL

Mps were determined in open capillary and are uncorr. IR spectra were recorded in nujol mull. PMR spectra were measured with TMS as an internal standard. MS were measured at 70 eV. TLC was on Si gel G developed with BuOH–AcOH–H_2O (4:1:2).

Isolation of buchananine. Dried and milled stems (2 kg) were defatted with petrol (60–80°) and extracted with EtOH under reflux in a Soxhlet for 16 hr. The EtOH extract was concd under red. pres. and the viscous residue was triturated successively with $CHCl_3$, Me_2CO and 50% aq. MeOH. The aq. MeOH fraction on concn gave buchananine (0.18 g) which was crystallized from aq. MeOH, mp 137–38°; R_f 0.43; MS *m/e* (rel. int.): 223 (1.1), 149 (5.9), 136 (0.2), 124 (13.4), 123 (100), 106 (54), 105 (83.7), 78 (85), 77 (47). (Found: C, 50.84; H, 4.09; H, 4.83. $C_{12}H_{15}NO_7$ requires: C, 50.52; H, 5.26; N, 4.91%).

Hydrolysis of buchananine. The alkaloid (0.08 g) was refluxed with 1 N HCl in aq. MeOH (5 ml) for 10 min. The reaction product gave only 2 spots on TLC at R_f 0.31 and 0.64. MeOH was removed and the residue extracted with Et_2O. The hydrolysis products remained in the aq. layer which was evapd to dryness under red. pres. The vacuum dried mass was treated with Ac_2O (2 ml) and triethylamine (2 drops) and was kept overnight at room temp. The reaction mixture was poured over crushed ice and extracted with Et_2O. The Et_2O extract gave glucose pentaacetate crystallized from C_6H_6, mp 128–30°; IR: 1750 cm^{-1} (acetyl carbonyl), no OH, C=C or aromatic absorbtion; PMR ($CDCl_3$): δ 1.98 (6H, *s*, —OAc), 2.04 (6H, *s*, —OAc), 2.08 (3H, *s*, —OAc), 4.1–4.28 (3H, *m*), 5.06 (1H, C–5 of glucose), 5.17 (2H, C–6 of glucose), 5.7 (1H, *d*, $J_{1,2}$ = 8.5 Hz); MS *m/e* (rel. int.): 331 (4.8), 317 (2.2), 271 (1.1), 242 (56), 211 (7.3), 169 (40.1), 157 (100), M^+ was not observed [18]. Identity of glucose pentaacetate was confirmed by mmp and co-TLC with an authentic sample. Glucose was identified by TLC after hydrolysis of the pentaacetate (Found: C, 49.07; 5.51. Calc for $C_{16}H_{22}O_{11}$: C, 49.23; H, 5.64%).

Nicotinic acid. Excess AcOH and triethylamine in the aq. mother liquor, after removal of glucosepentaacetate, were removed *in vacuo*. The residue was crystallized from MeOH as fine needles, mp 234–36°; M^+, 123; UV λ_{max}^{MeOH} nm (logϵ) 216 (3.93), 257 (sh) (3.37), 263 (3.48), 269 (sh) (3.35), typical of nicotinic acid [19]. There was no shift in acid. IR_{max}^{nujol} cm^{-1} 1700 (acid carbonyl), 1600 (pyridine ring); PMR (D_2O): δ 7.92 (1H, *t*, J = 7 Hz), 8.82–9.0 (2H, *m*), 9.15 (1H, *s*, broad with finer splitting). Identity was confirmed by mmp and co-TLC with authentic nicotinic acid. (Found: C, 58.27; H, 3.98; N, 11.31. Calc. for $C_6H_5NO_2$: C, 58.53; H, 4.06; N, 11.38%).

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