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(+)-CATECHIN 3-RHAMNOSIDE FROM *ERYTHROXYLUM NOVOGRANATENSE*

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Key Word Index—*Erythroxylum novogranatense*; Erythroxylaceae; (+)-catechin 3-*O*-α-L-rhamnopyranoside; ombuin 3-*O*-rutinoside; procyanidins B_1 and B_3.

Abstract—A new flavanol glycoside has been isolated from stems of *Erythroxylum novogranatense* and its structure has been elucidated on the basis of MS and ^{1}H NMR spectroscopy and hydrolytic studies as (+)-catechin 3-*O*-α-L-rhamnopyranoside. On a similar basis of chemical and spectroscopic evidence, the presence of ombuin 3-*O*-rutinoside has been established. Furthermore, the occurrence of procyanidin biflavanoids has been demonstrated by the characterization of B_1 and B_3 as the first representatives of B-type proanthocyanidins in the genus *Erythroxylum*.

INTRODUCTION

Cultivated coca plants belong to two distinct species of the genus *Erythroxylum*: *E. coca* Lam. and *E. novogranatense* (Morris) Hieron [1]. Coca has been used in folk medicine in South America both as a general stimulant and for various gastrointestinal ailments [2–4]. The chemistry of the cultivated coca plants has been reviewed [5] and phytochemical [6] and chemotaxonomic [7] aspects of the genus *Erythroxylum* have recently been adequately covered. However, chemical investigations on *Erythroxylum* are mainly confined to the alkaloids, but other constituents may prove to be chemotaxonomically very useful [7]. Thus, little attention has been paid to associated condensed tannins derived from flavan-3-ols. These precursors generally occur as free phenols in nature, but are also found as gallates [8]. Only some few flavan-3-ol glycosides have hitherto been isolated [9–15]. We now report the isolation and characterization of a new flavan-3-ol glycoside from *Erythroxylum novogranatense*.

RESULTS AND DISCUSSION

Examination of the phenolic metabolites of the stems of *Erythroxylum novogranatense* has provided some simple flavanoids which apparently represent the precursors of associated condensed tannins. Isolation of one of these in low yield (0.1%) from the methanol extract by counter-current distribution between ethyl acetate and water, followed by Sephadex LH-20 chromatography of the ethyl acetate-soluble portion, led to its characterization as (+)-catechin 3-*O*-α-L-rhamnopyranoside. The structure and stereochemistry of this novel flavanol glycoside (**1**) were assigned on the basis of hydrolytic studies and by ^{1}H NMR and mass spectrometry of the acetate (**2**).

In addition, (+)-catechin accompanied by traces of (−)-epicatechin was isolated and identified by direct chromatographic and spectroscopic comparison with an authentic sample. Procyanidin biflavanoids, also detected in the same natural source, will be discussed in another communication. However, amongst these procyanidins B_1 and B_3 have already been identified representing the first compounds of this type characterized in *Erythroxylum*. Proof of their structures was provided by spectroscopic methods exhibiting properties identical to those previously reported [16–18] and in close agreement with those of synthetic reference samples [19].

The basic skeletal structure of **1** was readily recognized from ^{1}H NMR data. Analysis of the ^{1}H NMR spectrum of the acetate **2** revealed *meta*-coupled doublets (A-ring) at δ6.58 and 6.63 (J = 2.2 Hz) in addition to an ABC-system typical of the three protons on ring B, while the 2,3-*trans* stereochemistry was indicated by the large coupling constant ($J_{2,3}$ = 8.0 Hz) of the heterocyclic protons (C-ring). The identity of the aglycone moiety as (+)-catechin was further confirmed by hydrolysis with 2 N HCl, while a positive Molisch test [20] of **1** indicated the presence of a carbohydrate moiety. On acid hydrolysis **1** furnished rhamnose, identified by co-chromatography with an authentic sample. The glycosidic nature of **1** was also supported by a negative aniline hydrogen phthalate test [21].

The EI mass spectrum of **2** showed the $[M+1]^+$ peak at *m/z* 731 consistent with the peak at *m/z* 748 observed in the CI mass spectrum using ammonia for ionization. Elemental analysis of **2** corresponded to the molecular formula $C_{35}H_{38}O_{17}$ in accordance with the proposed structure. The fragmentation pattern, characterized by loss of ketene and acetoxy radicals, supported the structure of the aglycone as indicated by the fragment at *m/z* 458, while the appearance of the prominent peak at *m/z* 273 was typical of acetylated 6-desoxyaldopyranoses [22]. Other significant fragments at *m/z* 213, 171, 151 and 111 derived from the sugar after fission of the glycosidic linkage and subsequent loss of acetoxy fragments [23].

The ^{1}H NMR spectrum of **2** compared with that of (+)-catechin penta-acetate showed shielding of heterocyclic protons (C-ring). The significant upfield shift (Δδ 1.3) of the δ3.96 multiplet in **2**, attributed to H-3, suggested that the sugar could be placed on the C-3 position. Since four phenolic acetoxy signals were

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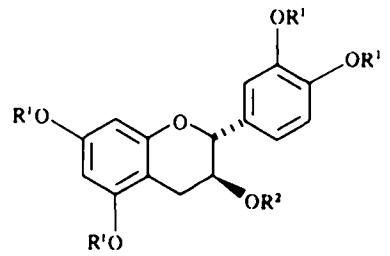
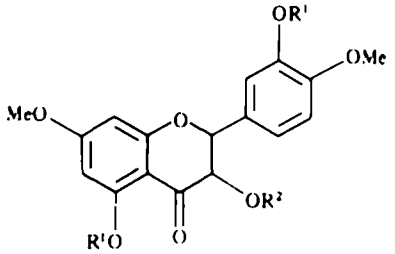


1 R^1 = H, R^2 = rhamnosyl
2 R^1 = Ac, R^2 = rhamnosyl (Ac)$_3$



3 R^1 = H, R^2 = rutinosyl
4 R^1 = Ac, R^2 = rutinosyl (Ac)$_4$

observed at δ2.25, 2.26 (× 2) and 2.33 in the ^{1}H NMR spectrum of **2** the carbohydrate moiety was shown to be attached to the aliphatic 3-hydroxyl group of (+)-catechin.

The carbohydrate moiety was defined by resonances at δ3.8–5.2 with integral intensities of five protons and a doublet at δ1.14 (J = 6.5 Hz) typical of the Me–CH system and hence immediate association with methyl pentoses. The α-anomeric configuration was assigned to the anomeric centre based upon the coupling constant (J = 1.5 Hz) of the anomeric proton signal at δ4.14 in the ^{1}H NMR spectrum of **2**. The above results were also consistent with the observation that **1** was not hydrolysed by almond emulsin but by hesperidinase. Starting with the equatorial H-1″ signal at δ4.14 the sequence of the protons of the pyranosyl unit readily followed from coupling constants. Thus, the H-2″ appeared as *dd* at δ4.98 ($J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 3.2 Hz) and a second *dd* at δ5.17 could be assigned to the H-3″ ($J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 10.0 Hz), while the H-4″ resonated as a triplet with $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz at δ5.00. The complex H-5″ was observed as two overlapping quartets (all J = 6.5 Hz), originating from coupling with Me-5″, while the individual quartet resonances were removed from each other by 10.0 Hz due to coupling with H-4″. The cited coupling constants illustrate the equatorial orientation of H-1″ and H-2″ and the axial arrangements of H-3″, H-4″ and H-5″, thus establishing the carbohydrate residue as α-L-rhamnose. Additional proof of the rhamnosyl unit was provided by comparison of the ^{1}H NMR spectrum of **2** with that of acetylated rhamnose.

This novel flavanol glycoside represents the first natural analogue of this rarely occurring group [9–15] possessing the carbohydrate moiety at the C-3 position, and also the first compound known hitherto within this group having a rhamnosyl unit. Although the isolation of 3-*O*-rhamnosides of 'leucodelphinidin' [24] and procyanidins B_3 and B_4 [25] has been claimed, these structures are not supported by satisfactory spectroscopic data.

Compound **1** is apparently accompanied by at least one other glycoside of unknown constitution, which, however, is considered to be dimeric. Due to the complexity of ^{1}H NMR spectra the structure could not be established conclusively. The occurrence of glycosylated biflavanoids is significant in that it supports the report on *O*-β-glucosides of procyanidin polymers [26]. However, it may be noted that the homogeneity and hence the purity of defined structures of polymers generally constitutes a problem in isolation procedures and is, therefore, very critical.

Another compound, isolated concurrently and identified as quercetin 7,4′-dimethyl ether (ombuin) 3-*O*-rutinoside (**3**), was significant in that it serves as taxonomic marker permitting differentiation between the two cultivated coca species [27]. Although ombuin 3-*O*-rutinoside has first been reported from *Phytolacca dioica* [28] and has also been characterized in *Erythroxylum novogranatense* [27] and *E. rufum* [29], only the recent communciation on its isolation from *E. argentinum* [30] provided spectroscopic evidence of the TMSi derivative of this rare flavonol glycoside.

The ^{1}H NMR spectrum of the acetate (**4**) similarly demonstrated the 5,7-disubstitution on ring A by the presence of *meta*-coupled doublets at δ6.52 and 6.82 (J = 2.2 Hz). The 3′,4′-substitution pattern on ring B was represented by an AMX-spin system located to low fields in the aromatic region (δ7.10–8.05), while the two aromatic methoxyls were clearly indicated by the singlets at δ3.90 and 3.92. The anomeric proton (H-1‴) of the glucopyranosyl unit appeared as a doublet at δ5.62 (J = 8.0 Hz) indicating direct attachment to the aglycone and β-configuration, respectively. The rhamnosyl unit of the disaccharide was characterized by a doublet at δ4.51 (J = 2.0 Hz) due to the anomeric proton (H-1⁗) and a highfield doublet at δ1.05 (J = 6.5 Hz), assigned to the rhamnose methyl group. The chemical shift of H-1‴ and the signals at δ3.3–4.4 and δ4.5–5.6, integrating for four and eight protons respectively, confirmed that the carbohydrate moiety was rutinose [31]. In addition to the above evidence, acid hydrolysis gave ombuin, glucose and rhamnose, and the UV spectra were in close agreement with those reported for ombuin 3-*O*-rutinoside [30].

Our present examination has enabled us to provide similar spectroscopic evidence for the occurrence of

ombuin 3-*O*-rutinoside in *Erythroxylum novogranatense*. Furthermore, **3** is present in both leaves and stems. The confirmed natural co-occurrence of ombuin 3-*O*-rutinoside with **1**, a new (+)-catechin glycoside and related tannins, may stimulate further studies on the genus *Erythroxylum*.

EXPERIMENTAL

NMR spectra were recorded at 300 MHz in $CDCl_3$ with TMS as internal standard. MS were obtained with a Varian MAT 44S Spectro System MAT 188 instrument. Acetylations were performed in Ac_2O–pyridine at room temp.

Isolation of compounds. Cultivated plant material of *E. novogranatense* (1.2 kg) was exhaustively extracted with MeOH and the combined extracts were evaporated *in vacuo* to dryness. The residue was dissolved in H_2O, washed with petrol and extracted with EtOAc, yielding a solid (11 g) on evaporation of the solvent. This portion was chromatographed on a column of Sephadex LH-20 using EtOH as eluting solvent. After the emergence of phenolic material, 15 ml fractions were collected: test tubes 40–50 afforded a mixture (81 mg) of (+)-catechin, (+)-catechin 3-*O*-α-L-rhamnoside and ombuin 3-*O*-β-rutinoside. Prep. TLC separation in EtOAc H_2O HCOOH (18:1:1) yielded the free phenols at R_f 0.8, 0.37 and 0.10, respectively.

Acetylation of the phenolic mixture and subsequent purification by prep. TLC in $CHCl_3$–EtOAc (3:2) gave their respective derivatives at R_f 0.65, 0.54 and 0.32. On further elution test tubes 107–128 contained procyanidin B_1, and 134–170 procyanidin B_3.

(+)-Catechin 3-O-α-L-rhamnopyranoside (**1**). The R_f 0.54 fraction (15 mg) was identified as the acetate **2**. (Found: C, 57.6; H, 5.4. $C_{33}H_{38}O_{17}$ requires C, 57.51; H, 5.25%); mp (uncorr.) 94–97°; $[\alpha]_{578}$ +2.17° ($CHCl_3$; *c* 0.23). EIMS *m/z* (rel. int.): 731 $[M+1]^+$ (20), 689 (18), 647 (28), 479 (34), 458 (93), 399 (16), 357 (16), 273 (52), 213 (15), 171 (24), 153 (100), 123 (40), 111 (96). CIMS (NH_3): 748 $[M+NH_4]^+$ (72), 706 (63), 664 (22), 526 (4), 484 (6), 418 (6), 308 (100), 273 (30), 213 (10), 153 (11). ^{1}H NMR ($CDCl_3$): δ1.14 (*d*, *J* = 6.5 Hz, Me-5″), 1.97 and 2.06 [all *s*, 3 × OAc (rhamnose)], 2.25, 2.26 (× 2) and 2.33 [all *s*, 4 × OAc (catechin)], 2.78 (*dd*, *J* = 9.0 and 16.0 Hz, H-4_{ax}), 2.91 (*dd*, *J* = 5.75 and 16.0 Hz, H-4_{eq}), 3.88 (2 × *q*, *J* = 6.5 and 10.0 Hz, H-5″), 3.96 (*m*, H-3), 4.14 (*d*, *J* = 1.5 Hz, H-1‴), 4.89 (*d*, *J* = 8.0 Hz, H-2), 4.98 (*dd*, *J* = 1.5 and 3.2 Hz, H-2″), 5.00 (*t*, Σ*J* = 20.0 Hz, H-4″), 5.17 (*dd*, *J* = 3.2 and 10.0 Hz, H-3″), 6.58 (*d*, *J* = 2.2 Hz, H-6), 6.63 (*d*, *J* = 2.2 Hz, H-8), 7.20–7.34 (*m*, 3H, H-2′, H-5′ and H-6′).

Compound **1** on hydrolysis with 2 N HCl gave rhamnose and an aglycone, extracted with EtOAc and identified as (+)-catechin by direct comparison with a reference sample. The aq. layer was examined by TLC with sugar standards. Enzymatic cleavage of **1** with hesperidinase was carried out in an acetate buffer (pH 5.8) at 37°. The presence of only rhamnose in the aq. hydrolysate and (+)-catechin in the EtOAc layer was similarly established by co-TLC.

Ombuin 3-O-β-rutinoside (**3**). UV λ_{max}^{MeOH} nm: 256, 265 (sh) and 354; + NaOMe: 267, 295 (sh) and 367; + $AlCl_3$: 267, 298 (sh), 358 and 390; + $AlCl_3$:HCl: 267, 298 (sh), 358 and 390. No shifts were observed on addition of NaOAc or NaOAc-H_3BO_3. Acid hydrolysis of **3** with 2 N HCl gave ombuin, glucose and rhamnose.

The acetate **4** was obtained from R_f 0.32 (10 mg). ^{1}H NMR ($CDCl_3$): δ1.05 (*d*, *J* = 6.5 Hz, Me-5″), 1.94, 1.96, 2.03, 2.04, 2.08 and 2.12 [all *s*, 6 × OAc (sugar)], 2.35 and 2.44 [2 × *s*, 2 × OAc (aglycone)], 3.35 (*m*, H-5″), 3.65 (*m*, 3H, H-5‴ and CH_2-6″), 3.90 and 3.92 (2 × *s*, 2 × OMe), 4.51 (*d*, *J* = 2.0 Hz, H-1‴), 4.94–5.35 (*m*, 6H, H-2″, H-3″, H-4″, H-2‴, H-3‴ and H-4‴), 5.62 (*d*, *J* = 8.0 Hz, H-1″), 6.59 (*d*, *J* = 2.2 Hz, H-6), 6.82 (*d*, *J* = 2.2 Hz, H-8), 7.10 (*d*, *J* = 8.8 Hz, H-5′), 7.71 (*d*, *J* = 2.2 Hz, H-2′), 8.05 (*dd*, *J* = 2.2 and 8.8 Hz, H-6′).

Procyanidins B_1 and B_3. Procyanidins B_1 and B_3, obtained from Sephadex LH-20, were acetylated and purified by prep. TLC in $CHCl_3$ Me_2CO (3:2) (R_f 0.39 and 0.36, respectively), exhibiting spectral properties identical to those previously reported [16, 18, 19].

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