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TRUE STRUCTURE OF TRIUMBOIDIN, A FLAVONE GLYCOSIDE FROM *TRIUMFETTA RHOMBOIDEA*

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Key Word Index—*Triumfetta rhomboidea*; Tiliaceae; triumboidin; structural revision; 5,4′-dihydroxy-6-*O*-β-D-xylopyranosyloxy-7-*O*-α-L-rhamnopyranosyloxyflavone; FAB-MS; ^{13}C NMR.

Abstract—The structure of triumboidin isolated from *Triumfetta rhomboidea* as scutellarein 7-*O*-L-arabinorhamnoside is inconsistent with the spectral data. Its true structure has been established as scutellarein 6-xyloside 7-rhamnoside. ^{1}H and ^{13}C NMR and FAB-MS data for scutellarein 7-*O*-α-L-rhamnoside have also been provided.

INTRODUCTION

The isolation of a new flavone glycoside, triumboidin from the leaves of *Triumfetta rhomboidea* was reported by Srinivasan and Subramanian [1] who characterized it as the 7-*O*-L-arabinosylrhamnoside of scutellarein and wrongly named it scutellarein-7-*O*-L-rhamnosylarabinoside based on products of hydrolysis and UV spectral data. However the λ_{max}^{MeOH} reported for triumboidin (238 sh, 277, 335 nm) was different from that of scutellarein (286, 335) [2], 7-*O*-methylscutellarein (285, 335) [2] and 7-*O*-rhamnosylscutellarein (238 sh, 286, 335) [1] and close to 6-*O*-methylscutellarein (275, 336) [2] and 6,7-di-*O*-methylscutellarein (277, 331) [2], indicating the involvement of the 6-hydroxyl in glycosylation. Also no evidence was provided for the presence of the disaccharide moiety. We therefore decided to reinvestigate its structure and the results are reported here.

RESULTS AND DISCUSSION

Triumboidin was reisolated from fresh leaves of *Triumfetta rhomboidea* and obtained as light yellow needles, mp 220–222°. It was indistinguishable from the sample [1] kindly provided by Srinivasan and Subramanian except in mp (198°). On treatment with 2 N HCl (MeOH medium, 100°, 2 hr) it yielded scutellarein, L-rhamnose and D-xylose in approximately 1:1:1 proportion. The identity of the sugars was established by co-chromatography with authentic markers using water saturated phenol and other developing solvents. H_2O_2 oxidation [3] of triumboidin gave only L-rhamnose and D-xylose, indicating it to be a diglycoside and not a bioside. On partial hydrolysis (2% H_2SO_4, 28°, 24 hr), triumboidin yielded a scutellarein monoside and D-xylose along with small quantities of scutellarein and L-rhamnose. The monoside was identical with the second glycoside [1] present in the plant and was fully characterized as 7-*O*-α-L-rhamnopyranosylscutellarein by enzyme hydrolysis, UV, ^{1}H and ^{13}C NMR and FAB-MS (see Experimental).

The methanol spectrum of triumboidin (275, 334 nm) in comparison with that of the aglycone, scutellarein (285, 335 nm) indicated [2] glycosylation at the 6-hydroxyl. The presence of 6-*O*-glycosylation was also supported by a discernible shoulder around 390 nm in the $AlCl_3$ as well as $AlCl_3$–HCl spectrum (this shoulder was absent in the monoside as well as 6-hydroxy- and 6-methoxyflavones). The absence of any shift of band II by NaOAc was indicative of the absence of free 7-hydroxyl, although this is a not very reliable indicator in 6-*O*-substituted flavones

[4]. The glycosylation of the 7-hydroxyl was further inferred [5] from the longer λ_{max} (392 nm) with NaOAc compared to NaOMe (382) as well as from the absence of band III in the NaOMe spectrum. The UV data thus revealed triumboidin as a scutellarein 6,7-diglycoside. ^{1}H NMR of triumboidin (see Experimental) indicated [6, 7] the sugar moieties as α-L-rhamnoside (H-1 at 5.53, *d*, J = 1 Hz; Me at 1.14, *d*, J = 6.1 Hz) and β-D-xyloside (H-1 at 4.98, *d*, J = 8.5 Hz). ^{13}C NMR data (see Experimental) on the monoside gave further support to the nature of the aglycone [8] as well as the 7-*O*-α-L-rhamnoside [9] moiety. The ^{13}C resonances of the sugar carbons were closely comparable to those reported for a 7-*O*-α-L-rhamnopyranosyloxyflavone [9]. Triumboidin is thus 6-*O*-β-D-xylosyl-7-*O*-α-L-rhamnopyranosylscutellarein, and is a new glycoside not previously known. Final proof of structure came from FAB-MS. In addition to the molecular ion at *m/z* 563 ([M − H]$^-$, 25%, negative ion FAB, thioglycerol) peaks corresponding to the loss of xylose (*m/z* 431, 10%) and rhamnose moieties (*m/z* 417, 15%) and both xylose and rhamnose (*m/z* 285, 30%) were observed. The pyranoside nature of xylose was suggested in the ^{1}H NMR signals as well as from the widespread occurrence of flavonoid xylopyranosides [10]. The material was insufficient for confirmation by ^{13}C NMR analysis [7].

Triumboidin did not undergo hydrolysis with takadiastase. It was also unaffected by a mixture of α-L-rhamnosidase and β-D-xylosidase whereas scutellarein 7-*O*-α-L-rhamnoside was hydrolysed. The non-hydrolysability of 6,7-diglycosides by enzyme might be attributed to steric hindrance. The 6-*O*-glycosides have been observed earlier [11] to resist enzyme hydrolysis and a similar effect was noted [12] on 7-*O*-glycosides of 8-methoxyflavone. The comparatively easy acid hydrolysis [11] of 6-*O*-glycosides explains the formation of the rhamnoside (and not the xyloside) as the monoside in partial hydrolysis.

EXPERIMENTAL

Fresh leaves of *T. rhomboidea* (voucher specimen deposited at Jawaharlal Institute) collected from Pondicherry were extracted ×3 with boiling 80% EtOH. The residue from the EtOH concentrate was partitioned using C_6H_6, Et_2O, EtOAc and MeCOEt. EtOAc and MeCOEt fractions were subjected to prep. TLC (silica gel, $CHCl_3$–MeOH, 3:1). The lower band (R_f 0.40) on elution with MeOH and recrystallization yielded triumboidin and the higher band (R_f 0.65) scutellarein 7-*O*-rhamnoside.

Triumboidin. Light yellow needles, mp 220–222°. UV λ_{max}^{MeOH} nm (intensity relative to most intense peak as 1.00): 240 sh, 275 (0.70), 334 (1.00); NaOAc: 274 (0.95), 338 (1.00), 396 (0.36); NaOMe: 274 (0.50), 300 sh, 382 (1.00); $AlCl_3$: 282 (0.57), 301 (0.55), 362 (1.00), 390 sh; $AlCl_3$–HCl: 285 (0.59), 301 (0.59), 357 (1.00), 390 sh. ^{1}H NMR (350 MHz, DMSO-d_6, internal standard TMS): δ 7.97 (*d*, J = 9 Hz, 2H, H-2′ and H-6′), 7.00 (*s*, 1H, H-8), 6.94 (*d*, J = 9 Hz, 2H, H-3′ and H-5′), 6.88 (*s*, 1H, H-3), 5.52 (*d*, J = 1 Hz, 1H, H-1 of rh), 5.15, 4.94 and 4.75 (OH of rh) 4.98 (*d*, J = 8.5 Hz, 1H, H-1 of xy), 5.30, 5.03, 5.00 (OH of xy), 3.91, 3.78, 3.50, 3.35 and 3.03 (rh and xy H) and 1.15 (*d*, J = 6.5 Hz, Me of rh). FAB-MS (negative ion FAB, thioglycerol) *m/z* (rel. int.): 563 [M − H]$^-$ (25%), 431 [563 − xylosyl residue + H] (10), 417 [563 − rhamnosyl residue + H] (12), 285 [aglycone − H] (30), 249 (53), 185 (40), 127 (100). PC (Whatman 1, 28°, ascending, R_f × 100): 16 (H_2O), 43 (15% HOAc), 78 (50% HOAc), 59 (BAW), 84 (phenol) and 59 (t − BAW).

H_2O_2 oxidation. Triumboidin (3 mg) in 0.5 ml MeOH containing 0.1 N NH_3 (0.02 ml) was treated with 30% H_2O_2 (0.1 ml) and kept overnight at room temp. The residual peroxide was decomposed with 5% Pd–C and the product treated with 0.01 ml 0.88 NH_3 and heated at 100° for 5 min. The resultant sugars were co-chromatographed in four solvents with authentic markers.

Scutellarein 7-O-rhamnoside. Light yellow needles, mp 230–232°. UV λ_{max}^{MeOH} nm: 232 sh, 286 (0.68), 334 (1.00); NaOAc: 286 (0.56), 383 (1.00); NaOMe: 274 (0.21), 378 (1.00); $AlCl_3$: 240 sh, 290 sh, 303 (0.65), 366 (1.00); $AlCl_3$–HCl: 240 sh, 288 sh, 303 (0.70), 361 (1.00). ^{1}H NMR (270 MHz): δ 12.75 (*s*, 1H, 5-OH), 10.46 (*s*, 1H, 4′-OH), 8.88 (*s*, 1H, 6-OH), 7.97 (*d*, J = 8.8 Hz, 2H, H-2′ and H-6′), 7.00 (*s*, 1H, H-8), 6.94 (*d*, J = 8.8 Hz, 2H, H-3′ and H-5′), 6.82 (*s*, 1H, H-3), 5.53 (*d*, J = 1 Hz, 1H, H-1″), 5.18 (*d*, J = 4.4 Hz, 1H, OH), 4.98 (*d*, J = 5 Hz, 1H, OH), 4.77 (*d*, J = 5.7 Hz, 1H, OH), 3.94, 3.81, 3.35 and 3.17 (1H each, H-2″, H-5″, H-3″ and H-4″) and 1.14 (*d*, J = 6.1 Hz, 3H, Me). ^{13}C NMR (67.89 MHz, proton noise decoupled, DMSO-d_6, δ relative to TMS): 182.23 (C-4), 164.11 (C-2), 161.11 (C-4′), 150.66 (C-7), 148.99 (C-9), 146.98 (C-5), 130.95 (C-6), 128.43 (C-2′ and 6′), 121.31 (C-1′), 115.95 (C-3′ and 5′), 105.68 (C-10), 102.40 (C-3), 99.27 (C-1″), 94.54 (C-8), 71.77 (C-4″), 70.13 (C-2″), 69.87 (C-3″ and 5″) and 17.79 (C-6″). FAB-MS *m/z* (rel. int.): 431 [M − H]$^-$ (100%), 285 [431 − rhamnosyl residue + H] (90). PC (R_f × 100, in the above mentioned solvents for triumboidin): 5, 23, 63, 69, 86 and 67.

Enzyme hydrolysis. Scutellarein monoside (2 mg) in 0.5 ml acetate buffer (pH 5.5) was mixed with the mixture of α-L-rhamnosidase and β-D-xylosidase (obtained from the mushroom *Coniophora puteana*) and kept at 30° for 2 hr. Almost completely hydrolysed to yield aglycone.

Scutellarein. Identified by UV, ^{1}H NMR, EIMS and co-chromatography with authentic sample.

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