Monochaetin, a Di-hyperin Ester of Tetrahydroxy-μ-truxinic Acid from *Monochaetum multiflorum*

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A new acylated flavonol glycoside, called monochaetin, was isolated along with three known flavonoids from the leaves of *Monochaetum multiflorum*, and its structure was characterized as a novel di-hyperin ester of 3,3′,4,4′-tetrahydroxy-μ-truxinic acid on the basis of chemical methods and 2D-NMR spectral analyses.

Key words *Monochaetum multiflorum*; Melastomataceae; monochaetin; acylated hyperin; flavonoid; tetrahydroxy-μ-truxinic acid

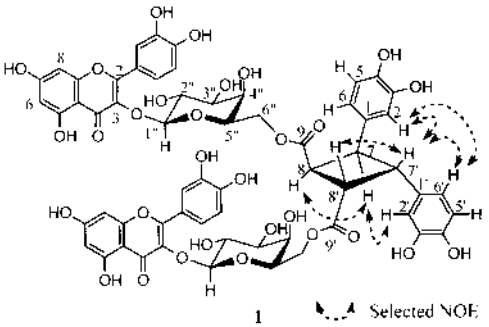
Melastomataceous plants, which are distributed in tropical and subtropical regions, are rich in polyphenols with diverse structures, particularly ellagitannins of large molecular weight.[1] In our continuing study of the polyphenolics of this family, we have isolated, in addition to hydrolyzable tannins characteristic of the Melastomataceae, a new acylated flavonol glycoside called monochaetin (**1**) from *Monochaetum multiflorum* (Bompl.) Naudin, a shrub endemic to Colombia. The shrub has been used as a treatment for infections and skin injuries in part of the country. This communication describes the elucidation of its unique structure (**1**), a di-hyperin ester of a cyclobutane-containing lignan, tetrahydroxy-μ-truxinic acid.

Chromatographic separation of the EtOAc extract obtained by liquid-liquid partition from a 70% aqueous acetone homogenate of the leaves gave four polyphenols of which the UV patterns on a photodiode array-detected HPLC[2] indicated their flavonoid nature. Three were identified as hyperin, isoquercitrin, and trifolin, respectively, by direct comparison with authentic specimens.

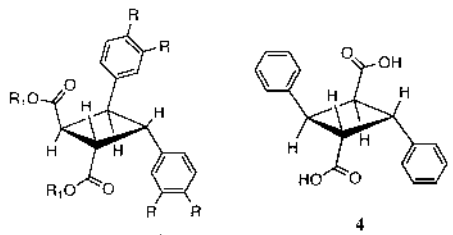
Monochaetin (**1**), in the form of a yellow amorphous powder, $[\alpha]_D$ +25.0° (c=1.0, MeOH), exhibited the UV absorption characteristic of a flavonol [λ_{max} nm (MeOH) 358 (4.61), 286 sh (4.46), 267 sh (4.67), 258 (4.71)]. Compound **1**, however, had a longer retention time on normal-phase HPLC[3] than those of the other flavonoids, suggesting that it has a larger molecular size than ordinary flavonoid glycosides. Monochaetin showed a pseudomolecular ion $[M+H]^+$ peak at *m/z* 1253 (49%) in electrospray ionization mass spectrometry (ESI-MS), and its molecular formula was determined to be $C_{60}H_{52}O_{30}$ by high-resolution (HR) ESI-MS (found: $[M+H]^+$ at *m/z* 1253.2689, Calcd 1253.2622). Its dimeric structure with two moles of hyperin (quercetin 3-*O*-galactoside) was indicated by MS fragmentation showing a successive loss of quercetin (Q) (*m/z* 951 $[M+H-Q]^+$ [43%], *m/z* 649 $[M+H-2Q]^+$ [19%]) as well as a base peak at *m/z* 303 $[Q+H]^+$, and by the ^{1}H- and ^{13}C-NMR spectra,[4] which included a pair of signals very similar to those of hyperin.[5]

Although the aliphatic proton signals in the ^{1}H-NMR spectrum were complicated due to overlapping of galactose signals and others, full assignments were achieved by a combination of ^{1}H–^{1}H shift correlation spectroscopy and ^{1}H J-resolved spectra measured at an elevated temperature (50 °C). Significant downfield shifts of the galactose C-6″ methylene protons relative to the corresponding signals of hyperin (δ 3.28→δ 3.52, 3.62; δ 3.45→δ 3.71, 3.84) were observed, indicating that both C-6″ hydroxyl groups of the galactosyl cores in **1** are acylated with a $C_{18}H_{14}O_6$ unit. The presence of a lignan moiety, a di-(3,4-dihydroxyphenyl)cyclobutane dicarboxyl group, as the acyl unit in the molecule was implied by two ester carbonyl carbon resonances (δ_C 171.0 and 171.2), two ABX-type aromatic proton signals, and an AA′BB′ system of methine proton signals [each ddd, ^3J=10.5 Hz (*cis*), ^3J=7.0 Hz (*trans*) and ^4J=2.0 Hz (long range)] which is characteristic of tetrasubstituted cyclobutane protons.[6] From the HMQC and DEPT spectra, the signals at δ_C 40.1, 40.4, 46.1, and 46.3 were assigned to the cyclobutane methine carbons. The HMBC spectrum of **1** revealed that the former two signals are attributed to carboxyl-bearing carbons, while the other two have 3,4-dihydroxyphenyl substituents. Based on these data, the acyl unit in **1** was assigned to either the μ-truxinyl-type ([7.7′,8.8′]-lignan) or α-truxillyl-type ([7.8′,8.7′]-lignan).[7,8] Of these, the former was preferred based on a comparison with the ^{1}H-NMR data reported for different configurational isomers of this type of lignan,[8] that is, the chemical shifts and coupling patterns of the cyclobutane methine protons of **1** were in agreement with those of μ-truxinic acid (**3**) (or its enantiomer), rather than those for α-truxillic acid (**4**). Furthermore, NOESY supported this assignment by clear NOE correlations among H-6/H-2′, H-2/H-6′, H-7/H-8, and H-7′/H-8′ which are only possible in truxinic acid series. The symmetrical structure of the parent acid with the cyclobutane ring was substantiated by the formation of an optically inactive methylated derivative (**2**)[9] upon methylation of **1** with trimethylsilyldiazomethane, followed by acid hydrolysis and methylation. Penta-*O*-methyl quercetin was also produced in the reaction.

Based on these data, the structure of monochaetin was established as **1**, and the absolute stereochemistry of the μ-



1 Selected NOE



2: R= OMe, R^1= Me
3: R= R^1= H

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truxinoyl moiety is under investigation.

Although numerous acylated flavonoid glycosides are known, only a few diflavonoid esters of dicarboxylic acid have been reported.[10,11] More notably, only stachysetin, which was isolated from *Stachys aegyptiaca* (Labiatae),[12] was previously recognized as a flavone glycoside truxinate ester. Monochaetin is thus the second example of such a diflavonoid ester.

References and Notes

1) Okuda T., Yoshida T., Hatano T., *Progress in the Chemistry of Organic Natural Products*, **66**, 1—117 (1996).
2) HPLC conditions: Column: Purospher RP-18 4.6 mm i.d.×250 mm, mobile phase: 0.01 M phosphate buffer–MeCN (85 : 15); oven temp. 40 °C; flow rate 1.0 ml/min.
3) Normal-phase HPLC conditions and retention times: Column; YMC Pack Sil A-003 5 μm, 4.6 mm i.d.×250 mm; mobile phase: *n*-hexane–methanol–THF–HCOOH (55 : 33 : 11 : 1 containing oxalic acid 450 mg per 1.0 l); room temp.; detector Shimadzu SPD-6A 280 nm, flow rate 1.5 ml/min. Trifolin t_R 4.1, isoquercitrin t_R 4.3, hyperin t_R 4.5, monochaetin 6.9 min.
4) Monochaetin (**1**) ^{1}H-NMR (500 MHz, DMSO-d_6, 50 °C) δ: quercetin moieties: 12.56 (2H, s, 5-OH), 6.19, 6.20 (1H each, d, J=2.0 Hz, H-6), 6.35, 6.37 (1H each, d, J=2.0 Hz, H-8), 7.51, 7.52 (1H each, d, J=2.0 Hz, H-2′), 6.79, 6.81 (1H each, d, J=8.5 Hz, H-5′), 7.58, 7.59 (1H each, dd, J=8.5, 2.0 Hz, H-6′); galactose moieties: 5.24, 5.26 (1H each, d, J=7.5 Hz, H-1″), 3.52 (3H m, 2×H-2″, H-6″), 3.29, 3.32 (1H each, dd, J=9.5, 3.0 Hz, H-3″), 3.40 (2H, br d, J=3.0 Hz, H-4″), 3.11, 3.12 (1H each, m, H-5″), 3.62 (1H, dd, J=11.5, 7.0 Hz, H-6″), 3.84 (1H, dd, J=10.5, 6.5 Hz, H-6″), 3.71 (1H, dd, J=11.5, 5.0 Hz, H-6″); μ-truxinoyl moiety: 6.52, 6.54 (1H each, d, J=2.0 Hz, H-2,2′), 6.56, 6.59 (1H each, d, J=8.0 Hz, H-5,5′), 6.25, 6.30 (1H each, dd, J=8.0, 2.0 Hz, H-6,6′), 3.87, 3.89 (1H each, ddd, J=10.5, 7.0, 2.0 Hz, H-7,7′), 3.46, 3.54 (1H each, ddd, J=10.5, 7.0, 2.0 Hz, H-8,8′). ^{13}C-NMR (125 MHz, DMSO-d_6, 50 °C) δ: quercetin moieties: 156.3 (2×C-2), 133.6, 133.7 (C-3), 177.4 (2×C-4), 161.3 (2×C-5), 98.8 (2×C-6), 164.1, 164.2 (C-7), 93.6 (2×C-8), 156.3 (2×C-9), 104.0 (2×C-10), 121.2 (2×C-1′), 116.1 (2×C-2′), 144.8 (2×C-3′), 148.5 (2×C-4′), 115.3 (2×C-5′), 121.8 (2×C-6′); galactose moieties: 101.9, 102.0 (C-1″), 71.0 (2×C-2″), 72.8, 72.9 (C-3″), 68.4, 68.7 (C-4″), 72.5, 73.0 (C-5″), 62.0, 63.3 (C-6″); μ-truxinoyl moiety: 129.5, 129.6 (C-1,1′), 114.8, 114.9 (C-2,2′), 144.8 (C-3,3′), 144.0, 144.1 (C-4,4′), 115.2, 115.5 (C-5,5′), 118.1, 118.4 (C-6,6′), 46.1, 46.3 (C-7,7′), 40.1, 40.4 (C-8,8′), 171.0, 171.2 (C-9,9′).
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9) Dimethyl 3,3′,4,4′-tetramethoxy-μ-truxinate (**2**): UV λ_{max} (EtOH) nm (log ε: 230 (3.66), 280 (3.16). ESI-MS *m/z*: 445 (18%) $(M+H)^+$, 413 (35%) $(M+H-MeOH)^+$, 223 (100%) (3,4-di-MeO–C_6H_3–CHCH–COOMe+H)$^+$, 163 (56%) $(C_{10}H_{10}O_2+H)^+$, 149 (34%) $(C_9H_9O_2)^+$. ^{1}H-NMR (500 MHz, $CDCl_3$, 27 °C) δ 6.81 (2H, d, J=2.0 Hz, H-2,2′), 6.83 (2H, d, J=8.0 Hz, H-5,5′), 6.86 (2H, dd, J=8.0, 2.0 Hz, H-6,6′), 4.38 (2H, dd, J=10.5, 7.5 Hz, H-7,7′), 3.90 (2H, dd, J=10.5, 7.5 Hz, H-8,8′), 3.37 (6H, s, 2×CH_3OCO–), 3.87, 3.90 (6H each, s, 4×CH_3O–).
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