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Iridoid Glucosides from *Myxopyrum smilacifolium*

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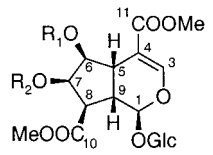
From a single leaf of a herbarium sheet of *Myxopyrum smilacifolium* was isolated the iridoid glucosides myxopyroside (**1**) and its 6-*O*-acetyl-7-*O*-(*E*/*Z*)-*p*-methoxycinnamoyl esters (**2**/**3**) of dimethyl forsythide. The presence of these compounds indicates a close relationship with the genus *Nyctanthes*, from which similar compounds have earlier been reported. This finding corroborates relationships recently found within the family Oleaceae by modern gene sequencing techniques.

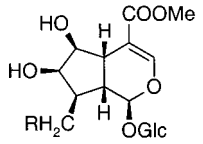
The genus *Myxopyrum* (Oleaceae) consists of four species from tropical and subtropical East Asia.[1] Chemical research on the genus appears to be limited to an investigation of the flavonoids.[2] In connection with a recent examination of the phylogeny of Oleaceae based on chloroplast DNA sequencing,[3] we have had occasion to analyze *Myxopyrum smilacifolium* Blume ssp. *confertum* (Kerr) Kiew for iridoids.

Only a single leaf (432 mg) from a herbarium sheet of *M. smilacifoilum* was available for chemical investigation. It was collected in Thailand in 1970 and kept in the Herbarium of the Botanical Museum, The University of Copenhagen (C). Extraction with ethanol followed by reversed-phase chromatography provided the iridoid glucosides, namely, pure **1** (5 mg) and an *E*/*Z*-mixture of **2** and **3** (7 mg).

The first compound (**1**) was named myxopyroside. HRFABMS showed the molecular composition to be $C_{18}H_{26}O_{13}$. The ^{13}C NMR spectrum (in D_2O) displayed the expected 18 signals (see Experimental Section), of which six could be assigned to a β-glucopyranosyl moiety. The remaining signals, including two methoxycarbonyl groups, suggested the aglucon to be a dihydroxy-substituted forsythide dimethyl ester[4] like that shown in the formula. The ^1H NMR spectrum was also in agreement with the structure **1**, the signals being assigned by a COSY spectrum, after which all ^{13}C NMR signals could be assigned by HSQC and HMBC experiments. Assuming the usual stereochemistry at C-1, C-5, and C-9, the remaining centers in **1** were determined to be the same as that of the known 6β-hydroxyloganin[5] (**4**) by finding almost the same set of $^3J_{H,H}$ coupling constants for the protons at C-5 through C-9 in the two compounds. Also, the ^{13}C NMR shifts seen for C-6 and C-7 (δ 78.8 and 73.3, respectively) in **1** proved the *cis*-disposition of the hydroxy groups when compared to the much larger values found for the 6β,7α-*trans*-substituted isomer (δ 84.3 and 85.8, respectively).[6] Finally, a NOESY spectrum confirmed that H-6, H-7, and H-8 were placed on the α-face of the molecule, since strong interactions were seen from H-1 to H-6 and H-8, and also from H-6 to H-8. In concert with this, no interactions were seen between H-9 and H-6 or H-7, and none between H-5 and H-7 or H-8, proving the structure for myxoporoside to be that given as **1** (6β,7β-dihydroxyforsythide dimethyl ester).

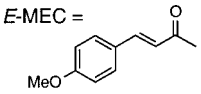


1 $R_1 = R_2 = H$
2 R_1 = Ac; R_2 = *E*-MEC
3 R_1 = Ac; R_2 = *Z*-MEC



4 R = H
5 R = OH

E-MEC =



Compounds **2** and **3** were isolated as an inseparable mixture of *E*/*Z*-isomers in the proportion 3:2. HRFABMS showed the molecular composition to be $C_{30}H_{36}O_{16}$. The NMR spectra (see Experimental Section) demonstrated that the compounds were ester derivatives of **1**. Besides a partly doubled set of signals from an iridoid similar to **1**, additional signals arising from an *E*/*Z*-mixture of *p*-methoxycinnamoyl (MEC) moieties as well as a set of doubled signals from an acetyl group could be seen. The uneven proportion of the two isomers as well as COSY and HSQC experiments allowed assignment of the signals to **2** and **3**, respectively. Since the $^3J_{H,H}$ coupling constants and the ^{13}C NMR signals arising from the iridoid moieties were very similar to those of **1** (allowing for the different solvent used), a common stereochemistry was indicated. In **2** and **3**, the large downfield shifts of H-6 (1.25 and 1.20 ppm, respectively) and H-7 (1.29 and 1.24 ppm) when compared to **1** demonstrated that these two positions were the sites of acylation. In an HMBC experiment, strong interactions were seen between two of the multiple signals around δ 171.8 (aliphatic C=O's) and δ 5.19/5.23 (H-6) and 1.95/1.98 (Ac-Me), proving the attachment of the acetyl groups to the 6-position in **2** and **3**. In the same experiment, similar interactions were seen between δ 168.7 (*E*-C=O) and δ 6.35 (*E*-H-α) and 5.70 (H-7), as well as between δ 167.5 (*Z*-C=O) and the two signals at δ 5.78 (*Z*-H-α) and 5.66 (H-7), confirming that a MEC group was esterified with the C-7 oxygen atoms in **2** and **3**. The structures of these are therefore 6β-acetyl-7β-(*E*/*Z*)-*p*-methoxycinnamoyl myxopyroside.

A similar leaf sample of *Dimetra craibiana* Kerr was also investigated, but no iridoids could be detected from this specimen.

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The presence of the compounds **1** and **2**/**3** in the genus *Myxopyrum* is of considerable chemotaxonomic interest. In a recent investigation of the phylogeny of the family Oleaceae,[3] the three genera *Nyctanthes*, *Dimetra*, and *Myxopyrum* were found to belong to the same clade (Myxopyreae). A number of iridoids have been reported from *Nyctanthes*,[7−14] most of which are aromatic mono- or diesters of loganin, 6*β*-hydroxyloganin (**4**), or nyctanthoside (**5**). It is therefore tempting to speculate that these compounds are on a common biosynthetic route: **4** → **5** → **1**, and this, together with the common ability to synthesize aromatic esters of the iridoids, indicates a close relationship between *Myxopyrum* and *Nyctanthes*.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova-500 instrument using the solvent peak (δ 4.75 or 3.31 for D_2O or MeOH-d_4, respectively) or the C-6′ peak (δ 61.5)[15] as the internal standard. HSQC, HMBC, and NOESY spectra were recorded on a Bruker Avance-400 instrument. HRFABMS (JEOL JMS-AX505W) were recorded in positive mode, using a bis(hydroxyethyl)disulfide matrix.

Plant Material. Single leaves were obtained from herbarium sheets. *Myxopyrum smilacifolium* ssp. *confertum* was collected in Thailand in February 1970; voucher: C. F. van Beusekom & T. Santisuk #2859 (C). *Dimetra craibiana* was collected in Thailand in October 1998; voucher: S. Suddee et al. #1000; The Forest Herbarium (BKF).

Workup of *M. smilacifolium*. A single dry leaf (432 mg) was crushed in a blender with sand (2 g) in EtOH (50 mL) and left to soak for 8 days. The mixture was filtered, and the extract taken to dryness (63 mg). Chromatography on a Merck Lobar Lichroprep RP-18 (size B) column eluting with H_2O−MeOH (7:2) gave myxopyroside (**1**; 5 mg). Elution with 1:1 provided an inseparable mixture of 6-*O*-acetyl-7-*O*-(*E*/*Z*)-*p*-methoxycinnamoylmyxopyroside (**2** and **3**; 7 mg).

Myxopyroside (1): colorless syrup; $[\alpha]^{21}_D$ −50° (*c* 0.4, MeOH); ^{1}H NMR (500 MHz, D_2O) δ 7.58 (1H, br d, J = 1 Hz, H-3), 5.37 (1H, d, J = 5.2 Hz, H-1), 4.41 (1H, dd, J = 4.0 and 5.5 Hz, H-7), 3.98 (1H, dd, J = 4.0 and 6.5 Hz, H-6), 3.81 (6H, s, 2 × OMe), 3.14 (1H, dd, J = 5.5 and 8.5 Hz, H-8), 3.09 (1H, br dd, J = 8.5 and 6.5 Hz, H-5), 3.02 (1H, dt, J = 5.2 and 8.5 Hz, H-9), 4.80 (1H, d, J = 8.0 Hz, H-1′), 3.95 (1H, dd, J = 6.0 and 12.5 Hz, H-6b′), 3.77 (1H, dd, J = 2.0 and 12.5 Hz, H-6a′), 3.54 (1H, t, J = 9.5 Hz, H-3′), 3.51 (1H, m, H-5′), 3.42 (1H, t, J = 9.5 Hz, H-4′), 3.32 (1H, dd, J = 8.2 and 9.5 Hz, H-2′); ^{13}C NMR (125 MHz, D_2O) δ 174.3 (C-10), 170.7 (C-11), 153.6 (C-3), 110.1 (C-4), 97.5 (C-1), 78.9 (C-6), 73.3 (C-7), 53.4 (OMe-10), 52.8 (OMe-11), 49.4 (C-8), 38.9 (C-9), 38.0 (C-5), 99.6 (C-1′), 77.1 (C-5′), 76.4 (C-3′), 73.3 (C-2′), 70.4 (C-4′), 61.5 (C-6′); HRFABMS *m*/*z* 473.1271 $[M + Na]^+$, calcd for $C_{18}H_{26}O_{13}Na$, 473.1221.

6-*O*-Acetyl-7-*O*-(*E*/*Z*)-*p*-methoxycinnamoylmyxopyroside (2 and 3): colorless syrup (proportion 3:2); ^{1}H NMR (500 MHz, MeOH-d_4) for **2** (*E*-form; major isomer) 7.53 (1H, d, J = 1.0 Hz, H-3), δ 5.70 (1H, dd, J = 4.5 and 7.0 Hz, H-7), 5.39 (1H, d, J = 6.0 Hz, H-1), 5.23 (1H, dd, J = 4.5 and 7.0 Hz, H-6), 3.70 (3H, s, OMe-11), 3.66 (3H, s, OMe-10), 3.3 (2H, obsc. H-5 and H-8), 3.09 (1H, ddd, J = 6.0, 8.0 and 9.0 Hz, H-9), 4.64 (1H, d, J = 8.0 Hz, H-1′), 3.92 (1H, dd, J = 2.5 and 12.0 Hz, H-6a′), 3.67 (1H, obsc., H-6b′), 3.2−3.4 (4H, obsc., H-2′−H-5′), 7.63 (1H, d, J = 16.0 Hz, H-*β*″), 7.58 (2H, d-like, J = 9.0 Hz, H-2″ and H-6″), 6.97 (2H, d-like, J = 9.0 Hz, H-3″ and H-5″), 6.35 (1H, d, J = 16.0 Hz, H-α″), 3.84 (3H, s, OMe-4″), 1.98 (3H, s, MeCOO−); ^{13}C NMR (125 MHz, MeOH-d_4) δ 171.8 (C-10*), 168.7 (C-11), 154.1 (C-3), 109.5 (C-4), 97.2 (C-1), 78.7 (C-6), 73.7 (C-7), 52.8 (OMe-10), 51.9 (11-OMe), 48.1 (C-8), 39.8 (C-9), 37.4 (C-5), 100.5 (C-1′), 78.5 (C-5′), 77.9 (C-3′), 74.6 (C-2′), 71.6 (C-4′), 62.8 (C-6′), 168.7 (C═O″), 163.4 (C-4″), 147.1 (C-*β*″), 131.2 (2C, C-2″ and C-6″), 128.1 (C-1″), 115.5 (2C, C-3″ and C-5″), 115.2 (C-α″), 55.9 (OMe-4″), 171.7 (Ac−C═O*), 20.7 (Ac-Me). For **3** (*Z*-form; minor isomer): ^{1}H NMR δ 7.51 (1H, d, J = 1.0 Hz, H-3), 5.66 (1H, dd, J = 4.5 and 7.0 Hz, H-7), 5.35 (1H, d, J = 6.0 Hz, H-1), 5.19 (1H, dd, J = 4.5 and 7.0 Hz, H-6), 3.70 (3H, s, OMe-11), 3.64 (3H, s, OMe-10), 3.3 (1H, obsc., H-8), 3.2 (1H, obsc., H-5), 2.95 (1H, ddd, J = 6.0, 8.0 and 9.0 Hz, H-9), 4.62 (1H, d, J = 8.0 Hz, H-1′), 3.91 (1H, dd, J = 2.5 and 12.0 Hz, H-6a′), 3.67 (1H, obsc., H-6b′), 3.2−3.4 (4H, obsc., H-2′−H-5′), 7.72 (2H, d-like, J = 9.0 Hz, H-2″ and H-6″), 6.99 (1H, d, J = 13.0 Hz, H-*β*″), 6.92 (2H, d-like, J = 9.0 Hz, H-3″ and H-5″), 5.78 (1H, d, J = 13.0 Hz, H-α″), 3.82 (3H, s, OMe-4″), 1.95 (3H, s, MeCOO−); ^{13}C NMR δ 171.8 (C-10*), 168.7 (C-11), 154.0 (C-3), 109.6 (C-4), 97.1 (C-1), 78.6 (C-6), 73.5 (C-7), 52.7 (OMe-10), 51.9 (OMe-11), 48.1 (C-8), 39.8 (C-9), 37.4 (C-5), 100.5 (C-1′), 78.5 (C-5′), 77.9 (C-3′), 74.6 (C-2′), 71.6 (C-4′), 62.8 (C-6′), 167.5 (C═O″), 162.3 (C-4″), 146.2 (C-*β*″), 133.5 (2C, C-2″ and C-6″), 128.6 (C-1″), 116.5 (C-α″), 114.5 (2C, C-3″ and C-5″), 55.8 (OMe-4″), 171.8 (Ac−C═O*), 20.8 (Ac-Me) (*uncertain assignment); HRFABMS *m*/*z* 675.1901 $[M + Na]^+$, calcd for $C_{30}H_{36}O_{16}Na$, 675.1845.

Workup of *Dimetra craibiana*. A single dry leaf (230 mg) was treated as above to give the extract (20 mg). However, no iridoids could be detected by ^{1}H NMR or HPLC.

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