94.7, 156.8, 105.6, 120.9, 113.2, 149.5, 146.9, 115.2, 122.4, 55.6, 101.0, 74.2, 76.4, 70.0, 75.9, 66.6, 100.7, 70.2, 70.5, 71.7, 68.1, 17.6, 98.3, 70.0, 70.2, 71.5, 69.7, 17.8.

Acid hydrolysis of **1–4**. A soln of **1** (100 mg) in 2 N HCl–MeOH was refluxed for 2 hr, neutralized with 3% KOH–MeOH and concd. The residue was subjected to silica gel CC eluting with $CHCl_3$–MeOH–H_2O (80:20:1) to afford isorhamnetin (37 mg) and methylsides of D-glucose (TLC solv. $CHCl_3$–MeOH–H_2O, 14:6:1). Compounds **2** (10 mg), **3** (6 mg) and **4** (5 mg) were hydrolysed in the same way as for **1** giving D-glucose and L-rhamnose and isorhamnetin.

Acetylation of **2–4**. **2**, **3** and **4** (5 mg of each) were separately acetylated with Ac_2O and pyridine (1:1) at room temp. overnight to give the corresponding peracetate **2a**, **3a** and **4a**. EIMS *m/z*: **2a**; 273, 316, 331, 400, **3a**; 273, 316, 400, 561, **4a**; 273, 316, 561.

Enzymic hydrolysis of **2** *and* **4**. A mixture of **2** (15 mg) and crude hesperidinase (20 mg) in HOAc–NaOAc buffer soln (pH 4.5, 1.5 ml) was incubated at room temp. for 1 min. MeOH was then added to the reaction mixture and evapd *in vacuo* to dryness to give a residue, the MeOH soluble part of which was subjected to silica gel CC eluting with $CHCl_3$–MeOH–H_2O (80:20:1) to yield **1** by TLC (R_f 0.51, solv. $CHCl_3$–MeOH–H_2O = 14:6:1). A small amount of **4** (5 mg) was hydrolysed with crude hesperidinase in HOAc–NaOAc buffer at room temp. for 3 min. The soln treated in the same way as **2** gave **3** by TLC (R_f 0.36, solv. $CHCl_3$–MeOH–H_2O = 14:6:1).

Isoeugenol β-D-glucopyranoside (**5**). Colourless needles, mp 170–174°, $[\alpha]_D^{25}$ −39.4° (pyridine; *c* 0.99), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1600, 1587, 1520 (phenyl), 1030 (OMe), 962 (C=C trans), 850 (phenyl). EIMS *m/z*: 164. ^1H NMR (C_5D_5N): 1.76 (3H, *d*, *J* = 6.0 Hz, 9-H_3), 3.74 (3H, *s*, OMe), 5.68 (1H, *d*, *J* = 7.0 Hz, glc 1-H), 6.08 (1H, *dq*, *J* = 6.0, 16.0 Hz, 8-H), 6.38 (1H, *d*, *J* = 16.0 Hz, 7- H), 6.95 (1H, *dd*, *J* = 2.0, 8.0 Hz, 6-H), 7.10 (1H, *d*, *J* = 2.0 Hz, 2-H), 7.48 (1H, *d*, *J* = 8.0 Hz, 5-H).

p-*Propenylphenol β-D-glucopyranoside* (**6**). Powder, $[\alpha]_D^{25}$ −55.0° (pyridine; *c* 1.0), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1605, 1508, 963, 840. EIMS *m/z*: 296 [M^+], 162, 134. ^1H NMR (C_5D_5N): 1.75 (3H, *d*, *J* = 6.0 Hz, 9-H_3), 5.60 (1H, *d*, *J* = 7.0 Hz, glc 1-H), 6.00 (1H, *dq*, *J* = 6.0, 16.0 Hz, 8-H), 6.35 (1H, *d*, *J* = 16.0 Hz, 7-H), 7.30 (4H, *m*, arom. H).

p-*Coumaryl alcohol β-D-glucopyranoside* (**8**). Colourless needles, mp 171–174°, $[\alpha]_D^{17}$ −57.4° (MeOH; *c* 0.94), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1603, 1518, 964. ^1H NMR (d_6-DMSO): 4.09 (2H, *t*-like, *J* = 5.1 Hz, 9-H_2), 6.24 (1H, *dt*, *J* = 5.1, 16.0 Hz, 8-H), 6.49 (1H, *d*, *J* = 16.0 Hz, 7-H), 6.98 (2H, *d*, *J* = 8.5 Hz, 2, 6-H), 7.35 (2H, *d*, *J* = 8.5 Hz, 3, 5-H).

Enzymic hydrolysis of **5**. A mixture of **5** (50 mg) and β-glucosidase (30 mg) in HOAc–NaOAc buffer (pH 4.5, 2 ml) was incubated at 37° for 1.5 hr. The reaction mixture was extracted with $CHCl_3$ and the $CHCl_3$ layer was evapd *in vacuo* to dryness to give isoeugenol by TLC and GLC (1.5% neopentylglycol succinate, column temp. 168°).

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EXTERNAL LEAF FLAVONOIDS OF *POLANISIA TRACHYSPERMA*

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Key Word Index—*Polanisia trachysperma*; Capparidaceae; leaf exudate; flavonoid aglycones.

Abstract—*Polanisia trachysperma*, a sticky annual weed, produces a terpenoid leaf resin. This exudate is shown to contain more than a dozen methylated flavonoid aglycones, some of which are rare natural products. Compounds with 6,8-dimethoxy substitution are predominant. *Polanysia trachysperma* is the first Capparaceae species found to accumulate external flavonoids.

INTRODUCTION

Polanisia trachysperma Torr. & Gray (Capparidaceae) is native to the central United States, but has spread widely elsewhere, growing in disturbed soil along roadsides, waste places, denuded areas and in sandy canyon washes or stream beds. It is an erect branching glandular hairy annual, which is very sticky and clammy to the touch [1].

It therefore attracted our interest in the scope of our continuing studies on flavonoid aglycones on plant surfaces [2] and indeed we have found a number of exudate flavonoids in this plant.

RESULTS AND DISCUSSION

The leaf exudate of *P. trachysperma*, obtained from air-dried aerial parts by rinsing with acetone, was processed by column chromatography as usual. It appeared to consist of mostly terpenoid material which was not studied further, but it also contained more than a dozen flavonoid aglycones. Eight of these were identified by TLC comparisons with markers and were found to be kaempferol and its 3-methyl ether, the 3-methyl, 3,3′-dimethyl and 7,3′-dimethyl ethers of quercetin, gossypetin 3,8-dimethyl ether, 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone (sideritiflavone), and 5,7,4′-trihydroxy-3,6,8,3′-tetramethoxyflavone. Four compounds were isolated in small amounts and according to their MS fragmentation they all were 8-methoxy- or 6,8-dimethoxy- flavonols ($M-15 > M^+$). Possible structures were deduced by evaluation of their chromatographic behaviour in conjunction with their molecular masses. By direct comparisons with authentic markers they were confirmed to be 5-hydroxy-3,6,7,8,4′-pentahydroxyflavone (5-hydroxyauranetin); 5,7,4′-trihydroxy-3,8,3′-trimethoxyflavone (gossypetin 3,8,3′-trimethyl ether); 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone; and 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone. The latter two compounds were further corroborated by their ^1H and ^{13}C NMR spectra (see Experimental).

Polanisia trachysperma thus exhibits in its leaf exudate several trivial flavonols, a methyl ether of 6,8-dihydroxyluteolin, a methyl ether of 6,8-dihydroxykaempferol, two of 8-hydroxyquercetin (gossypetin) and three of 6,8-dihydroxyquercetin. The 8-*O*- and 6,8-di-*O*-substituted compounds are rather rare natural products. Sideritiflavone was reported from *Sideritis* species, from *Mentha piperita*, several *Thymus* species, *Pteronia incana*. (Lamiaceae) and from two *Baccharis* species (Asteraceae). 5-Hydroxyauranetin was found earlier in *Citrus aurantia* (Rutaceae), *Calycopteris floribunda* (Combretaceae), *Hyptis tomentosa* (Lamiaceae) and *Digitalis viridiflora* (Scrophulasiaceae). Gossypetin 3,8-dimethyl ether, first isolated from *Cyanostegia microphylla* (Verbenaceae) and *Ricinocarpus muricatus* (Euphorbiaceae), was also found in *Enceliopsis nudicaulis*, *Geraea canescens*, in two *Gutierrezia* species and in some *Hemizonia* species (Asteraceae). Gossypetin 3,8,3′-trimethyl ether is so far known from *Cyanostegia angustifolia* (Verbenaceae), two *Cistus* species (Cistaceae), two *Gutierrezia* species, *Geraea canescens* and *Enceliopsis nudicaulis* (Asteraceae). The other three aglycones are even more rare. Thus, 5,7,4′-trihydroxy-3,6,8,3′-tetramethoxyflavone was first found in *Chrysothamnus viscidiflorus*, later in two *Gutierrezia* species and in *Gymnosperma glutinosa* (Asteraceae). 5,3′-Dihydroxy-3,6,7,8,4′-pentamethoxy flavone was previously reported from two *Gutierrezia* species (Asteraceae), while 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone was known only from *Blumea eriantha* (Asteraceae) and from *Citrus sinensis* (Rutaceae) (for references to plant sources see [3–5]).

With regard to the localization of these lipophilic flavonols it is quite clear that in the cited Asteraceae and Lamiaceae as well as in *Cistus* they are excreted and deposited on the leaf (and stem) surface [c.f. 6]. In *Citrus* they are accumulated in oil cavities of the fruit peel. However, nothing is known about the localization of these compounds in those species belonging to the Combretaceae (*Calycopteris*), Euphorbiaceae (*Ricinocarpus*), Scrophulariaceae (*Digitalis*), and Verbenaceae (*Cyanostegia*). The present results are important in so far as they allow us to add the Capparidaceae to the list of families known to accumulate external flavonoid aglycones [c.f. 2]. The joint occurrence of some of these polymethoxy flavonoids in several sources is also noteworthy.

EXPERIMENTAL

Polanisia trachysperma was collected in July 1985 in Santa Cruz Co., AZ *ca* 1 mile NE of Nogales at elev. 3920 ft. At this site it was growing with grasses on a disturbed gravel roadside in oak woodland. Aerial parts were air-dried in a paper bag. Voucher specimens (G. Yatskievych & T. Ranker, 85–252) are deposited at the Herbarium of Indiana University (IND) at Bloomington, Ind. and in the senior author's personal herbarium in Darmstadt.

On rinsing with Me_2CO, 229 g of plant material (containing a considerable proportion of stems) yielded 3.43 g of exudate material. Isolation of the flavonoid constituents was performed as described recently for *Pericome caudata* [7]. TLC fraction analysis and comparisons with markers were done on polyamide DC-11 (solvents: petrol 100–140–toluene–MeCOEt–MeOH, 12:6:1:1 and toluene–petrol–MeCOEt–MeOH, 12:6:2:1) and on silica gel (solvents: toluene–MeCOEt, 9:1 and toluene–dioxane–HOAc 18:5:1). Chromatograms were evaluated in UV_{366} before and after spraying with Naturstoffreagenz A. MS were run on a Varian MAT 311; NMR spectra were recorded on a Nicolet NTC 200 FT. Mps are uncorr. Authentic samples of gossypetin 3,8,3′-triOMe and of 5,3′-diOH-3,6,7,8,4′-pentaOMe were from *Gutierrezia microcephala* [8] and 5-OH-3,6,7,8,3′,4′-hexaOMe was obtained from the latter by methylation; 5-OH-3,6,7,8,4′-pentaOMe was from *Digitalis viridiflora* [9].

5-Hydroxy-3,6,7,8,4′-pentamethoxyflavone. Mp 124–125°. UV λ_{max}^{MeOH} nm: 337, 284, 228; + NaOH 405, 300; + $AlCl_3$ 365, 313, 288; + $AlCl_3$ + HCl 423 sh, 358, 312, 287. MS *m/z* (rel. int.) 388 (M^+, 79), 373 (M − 15, 100), 355 (5), 315 (5), 259 (4), 211 (9), 194 (9), 183 (10), 135 (18).

5,3′-Dihydroxy-3,6,7,8,4′-pentamethoxyflavone. Mp 169–170°. UV λ_{max}^{MeOH} nm: 350, 280, 260; + NaOH 395, 275; + $AlCl_3$ 377, 287, 275; + $AlCl_3$ + HCl 371, 288, 271. MS *m/z* (rel. int.) 404 (M^+, 86), 389 (M − 15, 100), 374 (7), 359 (4), 331 (7), 211 (7), 202 (9), 183 (5), 151 (11). ^1H NMR (200 MHz, DMSO-d_6): δ 12.46 (1H, *s*; 5-OH), 7.63 (1H, *dd*, *J* = 2 and 9 Hz; H-6′), 7.61 (1H, *d*, *J* = 2 Hz; H-2′), 7.15 (1H, *d*, *J* = 9 Hz; H-5′), 4.02, 3.90, 3.87, 3.82, 3.81 (3H each, *s*; 5 × OMe). ^{13}C NMR (50 MHz, DMSO-d_6): δ 155.9 (C-2), 138.0 (C-3), 178.7 (C-4), 148.1 (C-5), 135.5 (C-6), 152.4 (C-7), 132.5 (C-8), 144.4 (C-9), 106.8 (C-10), 122.2 (C-1′), 114.9 (C-2′), 146.5 (C-3′), 150.6 (C-4′), 112.1 (C-5′), 120.5 (C-6′), 59.7 (C-3-OMe), 60.6 (C-6-OMe), 61.9 (C-7-OMe), 61.5 (C-8-OMe), 55.7 (C-4′-OMe).

5-Hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone. Mp 113–114°. UV λ_{max}^{MeOH} nm: 348, 282, 261; + NaOH 405, 316, 298; + $AlCl_3$ 377, 292, 273; + $AlCl_3$ + HCl 420 sh, 371, 294, 270. MS *m/z* (rel. int.): 418 (M^+, 82), 403 (M − 15, 100), 388 (5), 373 (9), 345 (5), 211 (9), 209 (7), 183 (5), 165 (11). ^1H NMR (200 MHz, DMSO-d_6): δ 12.40 (1H, *s*; 5-OH), 7.75 (1H, *dd*, *J* = 2 Hz and 9 Hz; H-6′), 7.67 (1H, *d*, *J* = 2 Hz; H-2′), 7.22 (1H, *d*, *J* = 9 Hz; H-5′), 4.03, 3.91, 3.88, 3.86, 3.84, 3.82 (3H each, *s*; 6 × OMe). ^{13}C NMR (50 MHz, DMSO-d_6): δ 155.6 (C-2), 138.1 (C-3), 178.7 (C-4), 148.1 (C-5), 135.4 (C-6), 152.4 (C-7), 132.3 (C-8), 144.5 (C-9), 106.8 (C-10),

122.1 (C-1'), 111.8 (C-2'), 148.5 (C-3'), 151.4 (C-4'), 110.9 (C-5'), 122.0 (C-6'), 59.8 (C-3-OMe), 60.6 (C-6-OMe), 61.9 (C-7-OMe), 61.5 (C-8-OMe), 55.7 (C-3'-OMe), 55.4 (C-4'-OMe).

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NUMMULARINE-S: A CYCLOPEPTIDE ALKALOID FROM STEM BARK OF *ZIZYPHUS NUMMULARIA*

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Key Word Index—*Zizyphus nummularia*; Rhamnaceae; nummularine-S; 13-membered cyclopeptide alkaloid.

Abstract—From the stem bark of *Zizyphus nummularia* a 13-membered cyclopeptide alkaloid containing a short side chain has been isolated. The structure was determined by spectroscopic methods and chemical degradation. *Zizyphus nummularia* seems to be closely related to *Z. jujuba*, *Z. sativa* and *Z. amphibia*.

INTRODUCTION

The bark, leaves and fruit of *Zizyphus* species (Rhamnaceae) have been used as folk medicines in tropical and subtropical countries [1–3]. Chemical investigation of different *Zizyphus* species has led to the isolation of several cyclopeptide alkaloids [4, 5]. Such alkaloids are polyamide plant bases containing a styrylamine unit as an integral part of 13-, 14-, or 15-membered macrocyclic ring; the size of the rings has been adopted as the basis for classification of the compounds [4]. *Zizyphus juazeiro* [6], *Z. spina-christi* [7] and *Z. mauritiana* [8] possess only 14-membered cyclopeptide alkaloids, while *Z. abyssinica* [9] contains only 15-membered alkaloids of this class. 13- and 15-membered cyclopeptide alkaloids were isolated from *Z. mucronata* [10] and *Z. oenoplia* [11]. However, from *Z. nummularia* [12–16], just like *Z. sativa* [17–22], *Z. jujuba* [23] and *Z. amphibia* [24, 25] only 13- and 14-membered cyclopeptide alkaloids were isolated.

In continuation of our work on cyclopeptide alkaloids from the Rhamnaceae, we recently reported the isolation of several alkaloids from the stem bark of *Z. nummularia* [26]. We report herein the isolation and characterization of a further previously undescribed cyclopeptide alkaloid, nummularine-S (**1**) from the stem bark of this plant.

RESULTS AND DISCUSSION

The alkaloid (**1**) was isolated by consecutive TLC and chromatotron from the polar fractions of the CC. The IR spectrum showed typical absorptions for cyclopeptide alkaloids and the UV spectrum showed absorption maxima at 318 and 268 nm characteristic of the styrylamine moiety in 13-membered cyclopeptide alkaloids [27].

The molecular formula of (**1**) was determined by high resolution mass spectrometry as $C_{29}H_{36}N_4O_5$ ([M] + *m/z* 520.2693). The spectrum closely resembled that of nummularine-C (**2**), a 13-membered cyclopeptide alkaloid with a short side chain [12]. The main fragments observed in the mass spectrum are listed in Table 1 and the assignments are depicted in Scheme 1. The identity of each fragment was substantiated by high resolution mass spectrometry; the various fragments are represented as described in ref. [19]. The α-cleavage product **a** (*m/z* 86) of the terminal amino acid leucine or isoleucine formed the base peak of the spectrum and fragment **b** was found at *m/z* 463. Due to the short side chain, the ions **c**, **d**, **e** and **m** were absent [20]. The characteristic fragments for the methoxy styrylamine unit *m/z* 165, phenylalanine *m/z* 120 and hydroxyproline *m/z* 96 revealed the identity of the units forming the 13-membered heterocyclic ring of the