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FLAVONOIDS AND THE CHEMOTAXONOMY OF THREE *SOPHORA* SPECIES*

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Key Word Index—*Sophora* spp.; Leguminosae; chemotaxonomy; apigenin-7-*O*-rhamnosylglucoside-4′-*O*-glucoside; rhamnosylvitexin; flavone glycosides.

Abstract—*Sophora microphylla*, *S. prostrata* and *S. tetraptera* are distinguishable from one another by their leaf flavonoids. *S. microphylla* is distinguished by the present of rhamnosylvitexin and rhamnosylisovitexin and *S. tetraptera* by the presence of apigenin-7-*O*-rhamnosylglucoside-4′-*O*-glucoside and the 7-*O*-glucosides of apigenin, 7,4′-dihydroxyflavone, luteolin and 7,3′,4′-trihydroxyflavone. *Sophora prostrata* lacks all these flavonoids, but has several pigments which are common to all three species.

INTRODUCTION

A RECENT taxonomic study of species of New Zealand kowhai demonstrated that *Sophora microphylla* could be distinguished from the closely related *S. prostrata* and *S. tetraptera* by the PC patterns of the leaf flavonoids.[1] On this basis, and to some extent on the basis of the flavonoids of the seed coats, *Sophora* populations on the Chatham Is., Chile and Gough Is., were identified as *S. microphylla*, and infra-specific variations were cited in support of the theory that dispersal from New Zealand had led to the establishment of the other populations. The identification of the leaf flavonoids of each of the three species is the subject of the present communication.

DISCUSSION

The PC pattern of flavonoids from the leaves of *S. tetraptera* (Subfam. Lotoideae, tribe Sophoreae[2]) bears a striking resemblance to those obtained from the leaf extracts of certain *Baptisia*[3] and *Thermopsis*[4] species (tribe Podalyrieae[2]). A number of the flavonoids observed are common also to *S. microphylla* and *S. prostrata* and this was demonstrated by chromatography of the combined extracts of all three *Sophora* species (Fig. 1). Spots on the chromatogram in Fig. 1 labelled with numbers only are common to all 3 species, and those designated by letter, occur in visible quantities only in the species with that initial.

Compounds 4, 4′, 5, 5′, 6, 6′, and 7′, which are common to all three *Sophora* species and to *Baptisia lecontei*, were identified (see Table 1) in the present investigation by direct comparison with the known equivalent from *B. lecontei*.[3]

* Part II in the series "Chemotaxonomic Studies in Sophora". For part I see MARKHAM, K. R. and GODLEY, E. J. (1972) *N.Z. Jl Botany* **10**, 627.

[1] MARKHAM, K. R. and GODLEY, E. J. (1972) *N.Z. Jl Botany* **10**, 627.
[2] HEYWOOD, V. H. (1971) in *Chemotaxonomy of the Leguminosae* (HARBORNE, J. B., BOULTER, D. and TURNER, B. L., eds.), pp. 5, 23, Academic Press, London.
[3] MARKHAM, K. R. and MABRY, T. J. (1968) *Phytochemistry* **7**, 791.
[4] DEMENT, W. A. and MABRY, T. J. (1972) *Phytochemistry* **11**, 1089.

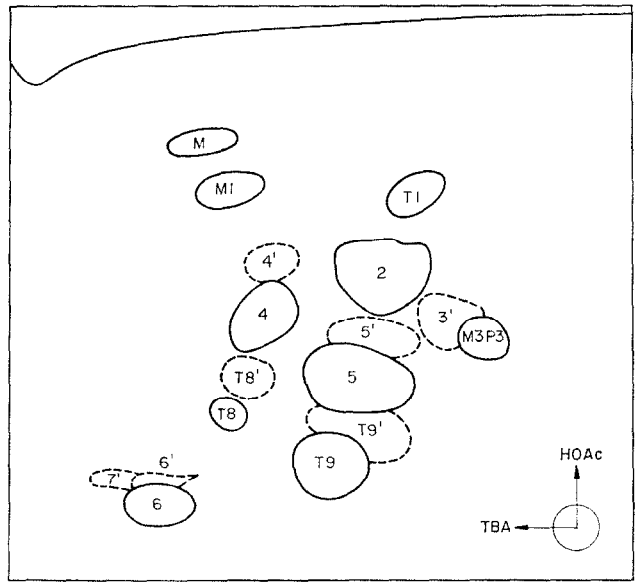


FIG. 1. PC OF COMBINED FOLIAGE EXTRACTS OF *Sophora microphylla*, *S. prostrata* AND *S. tetraptera*.

The only other major flavonoid common to all three species is compound 2. This was shown to be a 6,8-disubstituted apigenin glycoside[5] by PC, and UV and PMR spectroscopy. Its failure to hydrolyse or isomerize in acid suggests that it is a 6,8-di-*C*-glycoside in which

TABLE 1. PHYSICAL DATA ON COMPOUNDS DETECTED IN THE FOLIAGE EXTRACTS OF SOME *Sophora* SPECIES

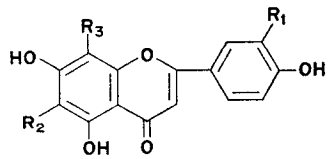
Spot No.	Spot colour* UV	UV/NH_3	R_f† TBA	HOAc	Structure or structural type
M	d	yg	0·56	0·80	Rhamnosylisovitexin
T1	d	d	0·30	0·65	Apigenin-7-*O*-rhamnosylglucoside-4′-*O*-glucoside
M1	d	yg	0·51	0·69	Rhamnosylvitexin
2	d	yg	0·35	0·53	Vicenin-2
M3/P3	d	y	0·17	0·37	Lucenin-2
3′	y.fl	y.fl	0·25	0·42	7,3′,4′-Trihydroxyflavone-6,8-di-*C*-glycoside?
4	d	yg	0·56	0·43	Apigenin-7-*O*-rhamnosylglucoside
4′	bl.fl	bl.g.fl	0·54	0·54	7,4′-Dihydroxyflavone-7-*O*-rhamnosylglucoside
5	d	y	0·41	0·30	Luteolin-7-*O*-rhamnosylglucoside
5′	bl.fl	yg.fl	0·37	0·38	7,3′,4′-Trihydroxyflavone-7-*O*-rhamnosylglucoside
6	d	y	0·74	0·06	Luteolin
6′	bl.fl	y.fl	0·74	0·1	7,3′,4′-Trihydroxyflavone
7′	bl.fl	yg.fl	0·87	0·12	7,4′-Dihydroxyflavone
T8	d	yg	0·62	0·24	Apigenin-7-*O*-glucoside
T8′	bl.fl	bl.g.fl	0·59	0·32	7,4′-Dihydroxyflavone-7-*O*-glucoside
T9	d	y	0·45	0·13	Luteolin-7-*O*-glucoside
T9′	bl.fl	yg.fl	0·39	0·19	7,3′,4′-Trihydroxyflavone-7-*O*-glucoside

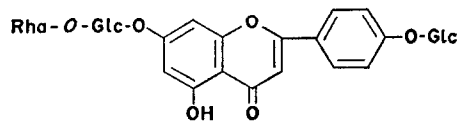
* Abbreviations as follows: UV—360 nm lamp; d—dark; y—yellow; yg—yellow-green; bl—blue; g—green; fl—fluorescent.

† R_f measured mainly from 2-D paper chromatograms.

[5] MABRY, T. J., MARKHAM, K. R. and THOMAS, M. B. (1970) *The Systematic Identification of Flavonoids*, Springer, New York.

the sugars at C-6 and C-8 are identical. It proved to be chromatographically and spectrally indistinguishable from vicenin-2(I).[6] Compound M3/P3, which was isolated in only trace amounts, was shown by similar means to be indistinguishable from lucenin-2(II).[7]





(III)

(I) R_1 = H ; R_2 = R_3 = glucosyl
(II) R_1 = OH; R_2 = R_3 = glucosyl
(IV) R_1 = R_2 = H ; R_3 = rhamnosyl-*C*-glucosyl
(V) R_1 = R_3 = H ; R_2 = rhamnosyl-*C*-glucosyl

The taxonomically more significant compounds however, are those that have only a limited distribution, i.e. T1, T8, T8′, T9, T9′, M and M1. Of the compounds characteristic of *S. tetraptera*, T8, T9, T8′ and T9′ were identified as the 7-*O*-glucosides of apigenin, luteolin and their 5-deoxy-equivalents respectively, by comparison with authentic material from *B. lecontei*.

The remaining *S. tetraptera* compound, T1, yielded apigenin, rhamnose and glucose on hydrolysis. It had a UV spectrum which, with maxima at 266 and 315 nm, is only interpretable as an apigenin type if T1 is glycosylated at both the 7- and 4′-hydroxyl groups.[5] Data from spectral shifts in fact confirmed this glycosylation pattern. The PMR spectrum revealed glucose and rhamnose C-1 proton signals at 5·08 and 4·55 ppm respectively, in the approximate ratio of 2:1. The chemical shift of the rhamnose C-1 proton indicates that the rhamnose is terminal.[5] Hydrolysis of T1 with β-glucosidase yielded a compound which was identical with compound 4 (apigenin-7-*O*-rhamnosylglucoside); thus T1 is apigenin-7-*O*-rhamnosylglucoside-4′-*O*-glucoside (III). This appears to be the first report of this compound as a natural product.

The two compounds, M and M1, which are characteristic of *S. microphylla* were isolated in only small quantities. Both gave the same apigenin-type UV spectrum and the spectral shifts indicated that all phenolic hydroxyl groups are unsubstituted. Acid hydrolysis of either compound produced rhamnose, together with two *C*-glucosylflavones which were indistinguishable from vitexin and isovitexin (the 8- and 6-*C*-glucopyranosyl derivatives of apigenin). It follows that M and M1 are the rhamnosylglycosides of isovitexin and vitexin, and since on mild hydrolysis M1 gave predominantly vitexin and M predominantly isovitexin, it is concluded that M1 is rhamnosylvitexin (IV) and M, rhamnosylisovitexin (V). Compound M1 cochromatographed with an authentic sample of rhamnosylvitexin.

From the identifications described above it would appear that *S. tetraptera* is distinguished from *S. microphylla* and *S. prostrata* primarily by the possession of a less effective rhamnosylating enzyme system. Thus flavonoids present in *S. tetraptera* as 7-*O*-glucosides (apigenin, luteolin, 7,4′-dihydroxyflavone and 7,3′,4′-trihydroxyflavone) appear in *S. microphylla* and *S. prostrata* only as the 7-*O*-rhamnosylglucosides. The 4′-glucosylation of apigenin-7-*O*-rhamnosylglucoside also appears to be distinctive of *S. tetraptera*. *S. microphylla* differs

[6] Chopin, J., Roux, B., Bouillant, M.-L., d'Arcy, A., Mabry, T. and Yoshioka, H. (1969) *Compt. Rend.* **268**, 980.
[7] Seikel, M. K., Chow, J. H. S. and Feldman, L. (1966) *Phytochemistry* **5**, 439.

from *S. prostrata* (and *S. tetraptera*) mainly in its ability to synthesize the *C*-glucosides, vitexin and isovitexin, and hence the rhamnosyl derivatives.

EXPERIMENTAL

Details of the origin of plant material and the extraction procedure are described in Ref. 1, and general chromatographic and spectral techniques are as outlined in Ref. 5. PMR spectra were measured on a Varian DA601 spectrometer fitted with a Varian C1024 time averaging computer. Chromatographic solvents were as follows: TBA = *t*-BuOH–HOAc–H_2O (3:1:1), HOAc = 15% aq. HOAc, MAW5 = MeOH–HOAc–H_2O (18:1:1), MAW10 = MeOH–HOAc–H_2O (8:1:1), 2% MC = 2% MeOH in $CHCl_3$. R_f for PC are listed in Table 1.

Standard hydrolysis conditions. Samples were refluxed for 1 hr with MeOH–3 N HCl (1:1).

Sugar analyses. By PC using as solvent, EtOAc–pyridine–H_2O (12:5:4), and aniline phthalate as spray reagent.

Isolation of compounds. Constituents of leaf extracts from both *S. microphylla* and *S. tetraptera* were initially separated by polyamide column chromatography. Final purification of most compounds was achieved by 1-D PC using as solvent TBA and/or HOAc.

Compounds 4, 4′, 5, 5′, 6, 6′, 7′, *T*8, *T*8′, *T*9 *and T*9′. Aglycones and glycosides were identified by comparisons of PC properties and UV spectra with those of authentic compounds. The aglycones were also compared by TLC (SiO_2, 2% MC). For data see Table 1 and Ref. 3.

Compound 2(*Vicenin*-2) *and M*3/*P*3 (*Lucenin*-2). *Physical data* (*compound* 2). λ_{max} (MeOH) 270, 295 sh, 332 nm; (NaOMe) 281, 330, 397 nm; (NaOAc) 278, 305 sh, 388 nm; ($AlCl_3$ and $AlCl_3$–HCl) 276, 298, 336, 375 sh nm; PMR signals of aromatic protons (d_6–DMSO) at 7·95 (*d*, *J* 8 Hz, H-2′,6′), 6·90 (*d*, *J* 8 Hz, H-3′,5′), 6·65 (*s*, H-3) ppm; identical to vicenin-2 on polyamide TLC, R_f 0·59 (MAW 5), and PC. *Physical data* (*M*3/*P*3). λ_{max} (MeOH) 258?, 268, 345 nm; (NaOMe) 268, 280 sh, 410 nm; (NaOAc) 270, 280 sh, 330 sh, 405 nm; (NaOAc–H_3BO_3) 262, 368, 430 sh nm; ($AlCl_3$) 272, 300 sh, 326 sh, 403 nm; ($AlCl_3$–HCl) 264, 272, 295 sh, 343, 383 sh nm; identical with lucenin-2 on polyamide TLC, R_f 0·51 (MAW 5), and PC.

*Compound T*1 [*apigenin*-7-O-*rhamnosylglucoside*-4′-O-*glucoside* (III)]. *Physical data.* λ_{max} (MeOH–H_2O) 266, 315, 337 sh? nm; (NaOMe) 281, 365 nm; (NaOAc) 267, 315 nm; ($AlCl_3$ and $AlCl_3$–HCl) 275, 293, 330, 380 nm. Signals in the PMR spectrum (d_6-DMSO) include, 8·03 (*d*, *J* 8 Hz, H-2′,6′), 7·2 (*d*, *J* 8 Hz, H-3′,5′), 6·96 (*s*, H-3), 6·80 (broad *s*, H-8), 6·47 (broad *s*, H-6), 5·08 (broad, glucose H-1), 4·56 (broad *s*, rhamnose H-1) ppm. Polyamide TLC R_f 0·71 (c.f. vicenin-1, R_f (0·54). The aglycone was purified by TLC (SiO_2, 4% MC) and was chromatographically (R_f 0·16, SiO_2, 2% MC) and spectrally (see spectra in Ref. 5) identical with apigenin. β-Glucosidase hydrolysis was carried out in dist. H_2O at 20° overnight, and the product was identical (PC, TBA and HOAc; and polyamide TLC R_f 0·66, MAW 10: λ_{max} (MeOH) 266, 332 nm; (NaOMe) 270, 300 sh, 348 sh, 387 nm; (NaOAc) 258 sh, 265, 350 sh, 387 nm) with compound 4.

*Compounds M and M*1 [*rhamnosylisovitexin* (V) *and rhamnosylvitexin* (IV)]. Both compounds had the same UV spectra: λ_{max} (MeOH) 268, 324 nm; (NaOMe) 278, 326, 393 nm; (NaOAc) 278, 300 sh, 377 nm; ($AlCl_3$ and $AlCl_3$–HCl) 275, 302, 340, 382 nm. Polyamide TLC: (MAW 10) R_fs (0·66 (M1), 0·66 (rhamnosylvitexin), 0·64 (xylosylvitexin); (MAW 5) R_fs 0·67 (M), 0·64 (M1 and rhamnosylvitexin). PC. (TBA and HOAc) M1 cochromatographed with rhamnosylvitexin. The flavonoids produced from M by acid hydrolysis were identical (PC and UV spectroscopy) to those produced from M1, although M yielded predominantly the high R_f compound, R_f 0·66 (TBA), 0·56 (HOAc), and M1 yielded predominantly the low R_f compound, R_f 0·46 (TBA), 0·33 (HOAc). They were interconvertible in acid and had the same UV spectra as did M and M1. The major product from M was indistinguishable (PC; polyamide TLC R_f 0·59, MAW 10; UV spectra) from isovitexin and that from M1 indistinguishable (as above but R_f 0·52) from vitexin.

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