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FLAVONOIDS OF NORTH AMERICAN SPECIES OF *THERMOPSIS*

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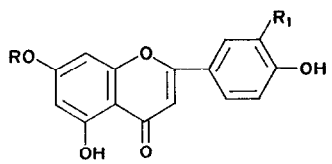
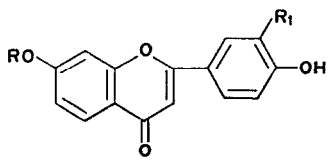
Abstract—The distribution of 12 flavones and 19 isoflavones in 13 taxa of *Thermopsis* is reported.

INTRODUCTION

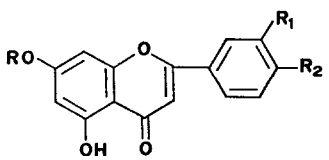
IN CONNECTION with our intensive biochemical systematic investigations of the legume genus *Baptisia*,[1] we have examined the flavonoid chemistry of the closely related genus *Thermopsis*. These two genera represent the only members of the tribe Podalyriaea which occur in North America. The *Thermopsis* flavonoid investigation was of particular interest because the flavonoid chemistry of *Baptisia* has been well established[1] and it has been suggested that *Baptisia* may have originated from a *Thermopsis*-like ancestor.[2]



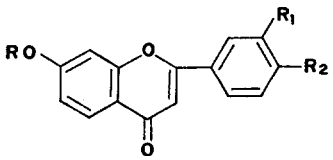
(I) R = glucosyl ; R_1 = OH
(IIa) R = rhamnoglucoside ; R_1 = OH
(IIb) R = glycoside ; R_1 = OH
(III) R = glucoside ; R_1 = H
(VI) R = glucoside ; R_1 = OCH_3



(IV) R = glucosyl ; R_1 = H
(V) R = glucosyl ; R_1 = OH



(VII) R = glucosyl ; R_1 = H ; R_2 = OH
(VIII) R = glucosyl ; R_1 = H ; R_2 = O-glucosyl
(X) R = glucosyl ; R_1 = H ; R_2 = OCH_3
(XII) R = glucosyl ; R_1 = OH ; R_2 = OH
(XIV) R = glucosyl ; R_1 = OH ; R_2 = OCH_3
(XIVa) R = glucosyl ; R_1 = OCH_3 ; R_2 = OH
(XV) R = H ; R_1 = OH ; R_2 = OCH_3
(XVa) R = H ; R_1 = OCH_3 ; R_2 = OH



(IX) R = glucosyl ; R_1 = H R_2 = OH
(XI) R = glucosyl ; R_1 = H R_2 = OCH_3
(XIII) R = glucosyl ; R_1 = OH R_2 = OCH_3

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[1] For a summary of references see: K. R. MARKHAM, T. J. MABRY and W. T. SWIFT, JR., *Phytochem.* **9**, 2359 (1970).

[2] B. L. TURNER, *Pure Appl. Chem.* **14**, 189 (1967).

TABLE 1. DISTRIBUTION OF FLAVONOIDS DETECTED IN *Thermopsis*

	Luteolin 7-*O*-glucoside (I)	Luteolin 7-*O*-rhamnoglucoside (IIa)	Luteolin 7-*O*-glycoside (IIb)[5]	Apigenin 7-*O*-glucoside (III)	4′,7-Dihydroxyflavone 7-*O*-glucoside (IV)	3′,4′,7-Trihydroxyflavone 7-*O*-glucoside (V)	Chrysoeriol 7-*O*-glucoside (VI)	Genistin (VII)	Genistein 4′,7-*O*-diglucoside (VIII)	Daidzin (IX)	Biochanin A 7-*O*-glucoside (X)	Formononetin 7-*O*-glucoside (XI)	Orobol 7-*O*-glucoside (XII)	Calycosin 7-*O*-glucoside (XIII)	Pratensein 7-*O*-glucoside (XIV)[6]	3′-*O*-Methylorobol 7-*O*-glucoside (XIVa)
T. montana																
Ariz, Apache Co.	●		○	●	○	○	○	●		○		●	●			
Colo, Chaffee Co.	●			●	○	○		●		○		●	●			
Colo, Gumnison Co.	●			●	○			●			●	●	●			
Colo, Las Animas Co.	●			●	○		○	●				●	●			
Colo, Saguache Co.	●		○	●	○	○	○	●		○		●	●	○	○	○
Colo, Saguache Co.	●			●	○	○	○	●				●	●			
N. Mex, Bernalillo Co.	●			●			○	●	○	○	●	●	●			
N. Mex, Sandoval Co.	●			●	○	○	○	●		○		●	●	○	○	○
N. Mex, Sandoval Co.	●	○		●	○	○	○	●		○	●	●	●	○	○	○
Utah, Garfield Co.	●			●	○	○	○	●		○		●				
Utah, Garfield Co.	●			●	○	○	○	●		○		●				
T. angustata																
Idaho, Owyhee Co.	●			●	○	○	○	●		○	●	●	●	○	○	○
Nevada, Elko Co.	●	●		●	○	○	○	●		○	●	●	●	○	○	○
Nevada, Elko Co.	●			●	○	○	○	●		○	●	●	●	○	○	○
Nevada, Elko Co.	●			●	○	○	○	●		○	●	●	●	○	○	○
T. montana var. ***ovata***																
Utah, Wasatch Co.	●	●		●	○	○	○	●			●	●				
T. divaricarpa																
Colo, Jefferson Co.	●			●	○	○	○	●		○		●	●			
Colo, Jefferson Co.	●			●	○	○		●		○		●	●			
Colo, Larimer Co.	●			●	○	○	○	●		○		●	●			
Colo, Larimer Co.	●			●	○	○	○	●		○		●	●			
Colo, Las Animas Co.	●			●	○	○	○	●		○		●	●			
Colo, Saguache Co.	●			●	○	○	○	●		○		●	●			
T. rhombifolia																
Colo, Boulder Co.	●			●	○	○	○	●		○		●	●			
Colo, Huerfano Co.	●			●	○	○		●		○		●	●			
Colo, Larimer Co.	●			●	○	○		●		○		●	●			
Colo, Saguache Co.	●			●	○	○	○	●		○		●	●			
T. gracilis																
Oregon, Josephine Co.	●			●	○	○	○	●			●	●			●	●
Oregon, Lane Co.	●			●	○	○	○	●		○		●	●			
T. argentea																
Calif, Modoc Co.	●		●	●	○	○	○	●			●	●				
Calif, Modoc Co.	●		●	●	○	○		●			●	●				
Calif, Modoc Co.	●		●	●	○	○		●			●	●				
Calif, Shasta Co.	●		●	●	○	○		●			●	●				
Calif, Siskiyou Co.	●		●	●	○	○	○	●			●	●				

	Luteolin 7-*O*-glucoside (I)	Luteolin 7-*O*-rhamnoglucoside (IIa)	Luteolin 7-*O*-glycoside (IIb)[5]	Apigenin 7-*O*-glucoside (III)	4′,7-Dihydroxyflavone 7-*O*-glucoside (IV)	3′,4′,7-Trihydroxyflavone 7-*O*-glucoside (V)	Chrysoeriol 7-*O*-glucoside (VI)	Genistin (VII)	Genistein 4′,7-*O*-diglucoside (VIII)	Daidzin (IX)	Biochanin A 7-*O*-glucoside (X)	Formononetin 7-*O*-glucoside (XI)	Orobol 7-*O*-glucoside (XII)	Calycosin 7-*O*-glucoside (XIII)	Pratensein 7-*O*-glucoside (XIV)[6]	3′-*O*-Methylorobol 7-*O*-glucoside (XIVa)
T. venosa																
Calif, Siskiyou Co.	●			●	○	○	○	●			●	●				
Oregon, Polk Co.	●			●	○	○	○	●		○		●		○		
T. macrophylla																
Calif, Del Norte Co.	●			●	○	○	○	●		○			●	●	○	
T. macrophylla var. *agnina*																
Calif, Santa Barbara Co.	●			●	○	○	○	●		○		●	●	○		
T. mollis																
Georgia, Walker Co.	●			○			○	●				●				
Georgia, Walker Co.	●		●	○	○	○	○	●	●	○		●	●			
N. Carolina, Chatham Co.	●		●	○	○	○	○	●	●	○		●	●			
T. fraxinifolia																
N. Carolina, Yancey Co.	●			●	○	○	○	●	●	○		●	●			
S. Carolina, Pickens Co.	●			●	○	○	○	●	●	○		●	●			
T. villosa																
N. Carolina, Macon Co.	●		●	●	○	○	○	●	●	○		●				
N. Carolina, Macon Co.	●		●	●	○	○	○	●	●	○		●				

Key: ●—major; ○—minor. In all cases, the various flavonoid glycosides were accompanied by the corresponding aglycones.

In the course of the present study three new flavonoids were identified: 3′-*O*-methylorobol, 3′-*O*-methylorobol 7-*O*-β-D-glucoside and genistein 4′,7-*O*-β-D-diglucoside. The flavone chrysoeriol and its 7-*O*-β-D-glucoside (VI) were found for the first time in the Leguminosae.

RESULTS

Distributional data for the 29 major flavonoids (12 flavones and 19 isoflavones) in 44 populations representing 13 taxa of the genus *Thermopsis* are presented in Table 1. Each compound listed was isolated and characterized from at least one *Thermopsis* species. Since the majority of the compounds had previously been isolated from *Baptisia*, their identity could readily be established by UV spectroscopy, hydrolysis and co-chromatography with known flavonoids.

3′-O-*Methylorobol* (XVa) *and* 3′-O-*methylorobol* 7-O-*glucoside* (XIVa). The aglycone material, which was isolated from a methanol–water (17:3) extract of *T. montana* by

polyamide chromatography followed by sephadex LH-20 column chromatography, showed UV spectral properties and R_fs similar to those reported for the isoflavone pratensein (XV)[3] and the NMR spectrum of the trimethylsilyl ether of the aglycone material indeed indicated the presence of a mixture of pratensein and 3′-*O*-methylorobol: A-ring protons sharp doublets ($J = 2{\cdot}5$ c/s) at 6·2 ppm and 6·4 ppm; two H-2 signals almost overlapped at 7·7 ppm; typical B-ring signals at 6·7–7·2 ppm for two 3′,4′-oxygenated B-rings; and two sharp *O*-methyl signals at about 3·8 ppm as expected for a 1:1 mixture of pratensein and 3′-*O*-methylorobol.

The glycoside material, upon hydrolysis with β-glucosidase, gave material which was chromatographically and spectrally equivalent to the aglycone mixture. Moreover, the UV spectrum of the glycoside in sodium acetate showed no shift in the short wavelength band, thus indicating the presence of a substituent at the 7-position.

Genistein 4′,7-O-β-D-*diglucoside* (VIII). Isolated from paper chromatograms of a methanol–water (7:3) extract of *T. mollis*, R_fs in TBA, 0·22 and in HOAc, 0·79. UV spectral analyses indicated the presence of both 7- and 4′-substituents and acid and enzyme hydrolyses gave genistein. The NMR spectrum of the trimethylsilyl derivative of (VIII) confirmed the genistein substitution pattern and the presence of two glucose moieties.

DISCUSSION

Although the uniformity of the flavonoid patterns throughout the genus *Thermopsis* indicated that flavonoids may be of only little value in determining interspecific relationships, the data do suggest that a comparison of the flavonoid distributions in *Thermopsis* and *Baptisia* could aid in establishing evolutionary sequences within the latter group, especially since it has been suggested that *Thermopsis*-like ancestors may have given rise to *Baptisia*. Utilizing only the flavonoid patterns in *Baptisia*, Harborne suggested the following phylogenetic sequence: *B. alba* group (flavones absent) → *B. leucophaea* group (flavone 7-glucosides) → *B. sphaerocarpa* group (flavone 7-rutinosides).[4] This sequence is based on the assumption that the presence of flavones is a more advanced character than the presence of flavonols and that complex glycosylation patterns represent an advanced character.

Turner proposed a similar phyletic sequence in *Baptisia* based on pod morphology.[2] If this sequence were valid, one might expect to find a similarity in the flavonoid patterns of *B. alba* and certain *Thermopsis* species; however, this is not the case. *B. alba* is characterized by the presence of flavonols and flavonol glycosides while all of the *Thermopsis* species were found to contain flavones, flavone glycosides, isoflavones and isoflavone glycosides. The latter are, nevertheless, observed in other *Baptisia* species. A re-evaluation of the relationship of *Baptisia* to *Thermopsis*, utilizing both chemical and morphological data is at present underway.

EXPERIMENTAL

Most of the plant material utilized in this study was collected by Dr. B. L. Turner in the spring of 1968 and 1970. Voucher specimens from each population are deposited in the University of Texas at Austin Herbarium.

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

[4] J. B. HARBORNE, in *The Leguminosae* (edited by J. B. HARBORNE, D. BOULTER and B. L. TURNER), Academic Press, London and New York (1971).

[5] Sugar substituent not identified.

[6] The pairs of compounds 23 and 23a, and 24 and 24a were isolated as 1:1 mixtures which were not separable by the chromatographic techniques employed.

Extractions were made with MeOH–H_2O (17:3) at room temp. for at least 24 hr. The majority of the compounds were isolated from *T. montana* by polyamide column chromatography using $CHCl_3$–MeOH–MeCOEt (12:2:1). Further purifications were made by sephadex LH-20 column chromatography with methanol as a solvent and by paper chromatography.

3′-*O*-Methylorobol (XVa) was isolated as a 1:1 mixture with pratensein by sephadex column chromatography in methanol; R_fs 0·83 in *t*-BuOH–HOAc–H_2O (3:1:1), 0·27 in 15% HOAc, 0·46 in benzene–HOAc–H_2O (6:7:3, top); λ_{max} (MeOH) 262, 288sh nm; (NaOMe) 273, 320sh nm; ($AlCl_3$ and $AlCl_3$/HCl) 272, 310sh 376 nm; (NaOAc) 271, 326 nm; (NaOAc/H_3BO_3) 262, 288sh nm. 3′-*O*-Methylorobol 7-*O*-glucoside (XIVa) was isolated (presumably as a mixture with pratensein glucoside, (XIV)) by paper chromatography from extracts of *T. montana*: R_fs 0·65 in *t*-BuOH–HOAc–H_2O (3:1:1), 0·68 in 15% HOAc; λ_{max} (MeOH) 260, 288sh nm; (NaOMe) 246sh, 256sh, 290sh nm; ($AlCl_3$ and $AlCl_3$/HCl) 271, 378 nm; (NaOAc) 260, 286sh nm; (NaOAc/H_3BO_3) 260, 286sh nm. Hydrolysis with 5% aqueous HCl at 100° for 30 min or with excess β-glucosidase in H_2O at 37° overnight gave a mixture of 3′-*O*-methylorobol and pratensein.

Genistein 4′,7-*O*-diglucoside was isolated by paper chromatography from *T. mollis*; R_fs 0·22 in *t*-BuOH–HOAc–H_2O (3:1:1), 0·79 in 15% HOAc; λ_{max} (MeOH) 259, 320sh nm; (NaOMe) 260, 344 nm; ($AlCl_3$ and $AlCl_3$/HCl) 273, 302sh 372 nm; (NaOAc) 259, 320sh nm; (NaOAc/H_3BO_3) 259, 320sh nm. Enzyme hydrolysis with β-glucosidase in H_2O at 37° for 1 hr. gave complete hydrolysis to authentic genistein.

When available, 10 plants from each population were individually analysed.

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Key Word Index—*Thermopsis* spp; Leguminosae; chemotaxonomy; flavones; isoflavones.