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BAPTISIA FLAVONOIDS: NUCLEAR MAGNETIC RESONANCE ANALYSIS*

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Abstract—The NMR characterization of several *Baptisia* flavonoids, including the 7-glycosides of luteolin, apigenin and orobol are discussed. NMR data for trimethylsilyl ethers of six additional flavonoids and two coumarins are reported.

INTRODUCTION

PRELIMINARY accounts recently reported the nuclear magnetic resonance analysis of trimethylsilyl ethers of flavonoid aglycones[1] and glycosides.[2] We now wish to discuss additional spectra of flavonoids and describe the application of the method for the characterization of some of the major flavonoids isolated from the genus *Baptisia* (family Leguminosae), luteolin, 5,7,3′,4′-tetrahydroxyflavone, apigenin, 5,7,4′-trihydroxyflavone, orobol, 5,7,3′,4′-tetrahydroxyisoflavone, and their 7-glycosides. The isolation and chemical characterization of flavonoids in *Baptisia* represents an extension of already well-documented biochemical systematic investigations of the genus. Extensive two-dimensional paper chromatograms of methanol extracts of various *Baptisia* plants disclosed over 100 phenolic substances including many flavonoids, nearly all of which could be used singly or in combination for systematic purposes.[3] For example, luteolin, apigenin and their glycosides are widespread in the genus and the variation in their relative concentrations in different species is systematically significant. A detailed analysis of these flavones was therefore of importance. It was often possible to distinguish by NMR of the carbon tetrachloride-soluble trimethylsilyl ether derivatives of flavonoid glycosides not only the hydroxyl substitution pattern of the flavonoid nucleus but also the position of glycosidation and the nature of the sugar moiety.[2]

RESULTS AND DISCUSSION

As previously noted,[1,2,4] the protons of the A-ring of flavonoids with the usual 5,7-hydroxylation pattern give rise to two doublets ($J_{meta} = 2{\cdot}5$ c/s) at about 6·0–6·6 ppm from tetramethylsilane. However, there are small but predictable variations in the chemical shifts of the C-6 and C-8 proton signals depending on the 5- and 7-substituents. In the spectra of

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[1] A. C. WAISS, JR., R. E. LUNDIN and D. J. STERN, *Tetrahedron Letters*, No. 10,513 (1964).

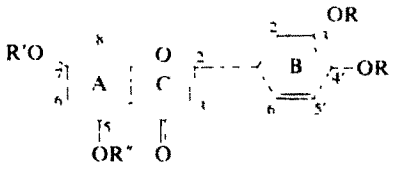
[2] T. J. MABRY, J. KAGAN and H. RÖSLER, *Phytochem.* **4**, 177 (1965).

[3] (*a*) R. E. ALSTON and B. L. TURNER, *Proc. Nat. Acad. Sci.* U.S. **48**, 130 (1962); (*b*) R. E. ALSTON, T. J. MABRY and B. L. TURNER, *Science*, **142**, 545 (1963).

[4] Leading references:
(*a*) J. MASSICOT, J. P. MARTHE and S. HEITZ, *Bull. Soc. Chim. France*, 2712 (1963); (*b*) T. J. BATTERHAM and R. J. HIGHET, *Australian J. Chem.* **17**, 428 (1964).

the four luteolin derivatives, II[2] and III-V (Fig. 1) which vary only in the C-5 and C-7 substituents, the B-ring protons display practically identical signal patterns between 6·6 and 7·5 ppm. The 5-trimethylsiloxy group in flavone and flavonol derivatives is often hydrolyzed when the derivatives are exposed to a moist atmosphere. It is therefore advantageous to take the spectra immediately after preparation of the trimethylsilyl derivative. Should hydrolysis occur, the 5-hydroxyl group, hydrogen bonded with the carbonyl, can be detected by its proton signal near 13 ppm. The spectrum of 7,3′,4′-tri-trimethylsiloxy-luteolin, III, is typical (Fig. 1). The C-3 proton signal is shifted downfield while the C-8 proton signal is shifted upfield, each about 10 c/s from their positions in the spectrum of II,[2] the totally trimethylsilylated luteolin. It is noteworthy that the signal of the C-6 proton (6·18 ppm) is unaffected by this hydrolysis of the ether group at C-5.

The protons on the flavonoid nuclei of glycosides and their aglycones produce similar NMR patterns. It is significant, however, that the A-ring proton signals appear downfield in the spectra of the derivatives of the *Baptisia* glycosides, luteolin-7-rhamnoglucoside, IV,



(I.) R, R′, R″ = H
(II) R, R′, R″ = $(CH_3)_3Si$
(III) R, R′ = $(CH_3)_3Si$, R′ = H
(IV) R, R = $(CH_3)_3Si$, R′ = (hexa-trimethylsilyl)-rhamno-β-glucosyl
(V) R, R = $(CH_3)_3Si$, R′ = (tetra-trimethylsilyl)-β-glucosyl

luteolin-7-glucoside, V, apigenin-7-glucoside, VI, and orobol-7-glucoside, VIII, with respect to the A-ring proton peaks in their respective aglycones[1,2] (Fig. 1). In contrast, the B- and C-ring proton signals for these glycosides compare closely with the corresponding signals in the aglycones. Thus glycosidation in the A-ring was indicated for IV, V, VI, and VIII. Further support for this conclusion is evidenced by the spectra of quercetin[1] and its C-3 glycosides, rutin,[2] quercitrin,[2] and hyperin (Table 1), in which all four display identical patterns for the A-ring protons. Furthermore, the signal for the A-ring proton at C-8 in the spectra of both penduletin, 5,4′-dihydroxy-3,6,7-trimethoxyflavone, and its 4′-glucoside, pendulin comes at 6·48 ppm in contrast to the downfield shift observed for the C-8 proton signal from irigenin-7-glucoside with respect to irigenin (Table 1).

The broad signal (one proton) near 5·0 ppm ($J \sim 7$ c/s) in the spectra of IV, V, VI, and VIII is characteristic for the axial–axial coupled proton at C-1 of a glucose attached at the 7-position in many flavonoids.[2] A β-linkage of glucose to the flavonoid is therefore demonstrated for these four *Baptisia* glucosides, confirmed for luteolin-7-glucoside by the easy hydrolysis with β-glucosidase. If, on the other hand, glucose or galactose is attached to the 3-position as in some flavonols, the C-1 proton appears as a sharp doublet near 5·7 ppm. For example, in hyperin, quercetin-3-galactoside, the galactose C-1 proton appears as a doublet at 5·63 ppm ($J = 7$ c/s) (Table 1) and in rutin, quercetin-3-rhamnoglucoside, the glucose C-1 proton comes at 5·73 ppm ($J = 7$ c/s).[2]

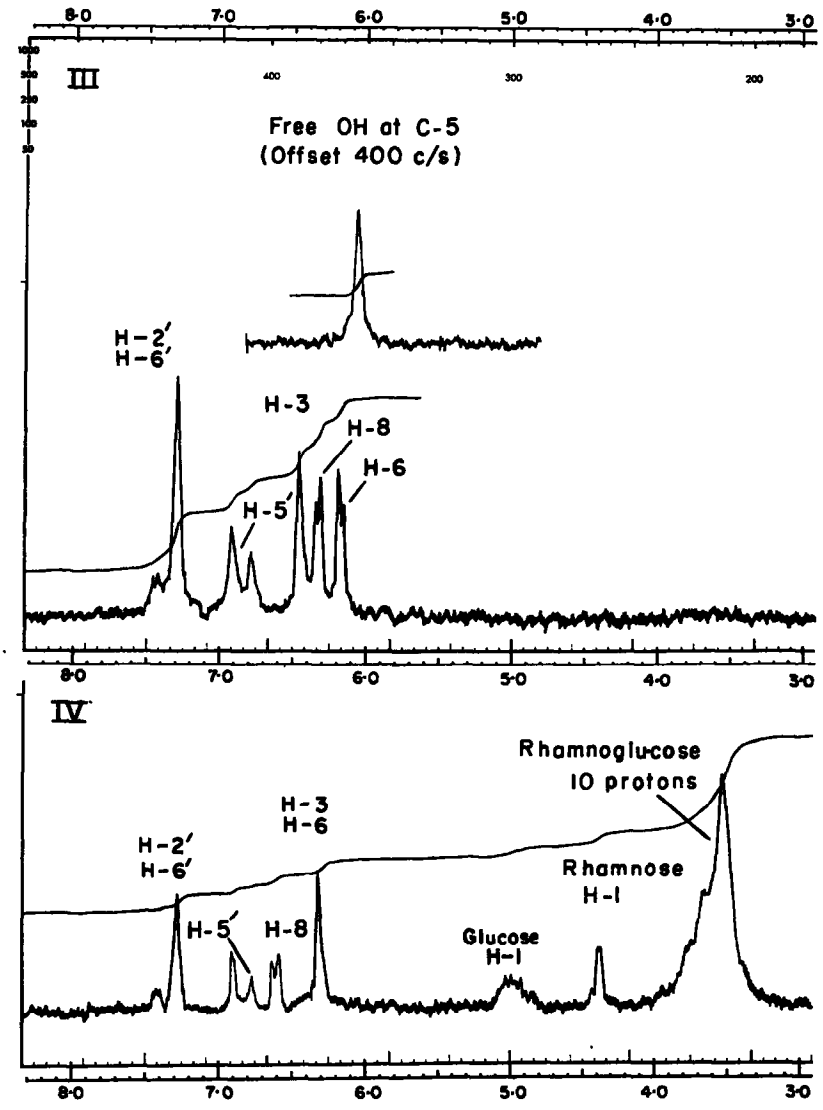


FIG. 1. NMR SPECTRA OF TRIMETHYLSILYL DERIVATIVES.

Luteolin with OH at C-5 free, III; luteolin-7-rhamnoglucoside, IV (methyl group of rhamnose comes at 0·8–1·0 ppm but is not shown here); luteolin-7-glucoside, V; apigenin-7-glucoside, VI; orobol, VII; orobol-7-glucoside, VIII.

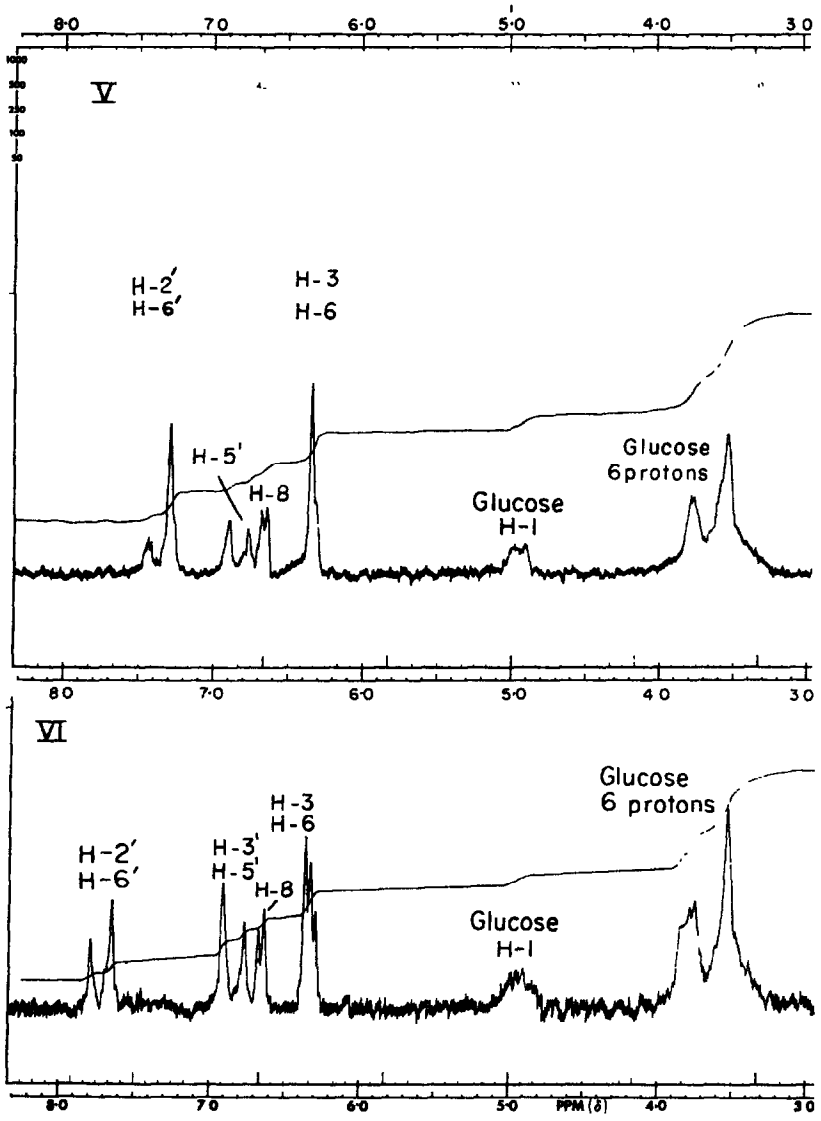

8·0
7·0
6·0
5·0
4·0
3·0
V
H-2′
H-6′
H-3
H-6
H-5′
H-8
Glucose
H-1
Glucose
6protons
VI
H-3
H-6
H-2′
H-6′
H-3′
H-5′
H-8
Glucose
H-1
Glucose
6 protons
PPM (δ)

FIG. 1. V and VI.

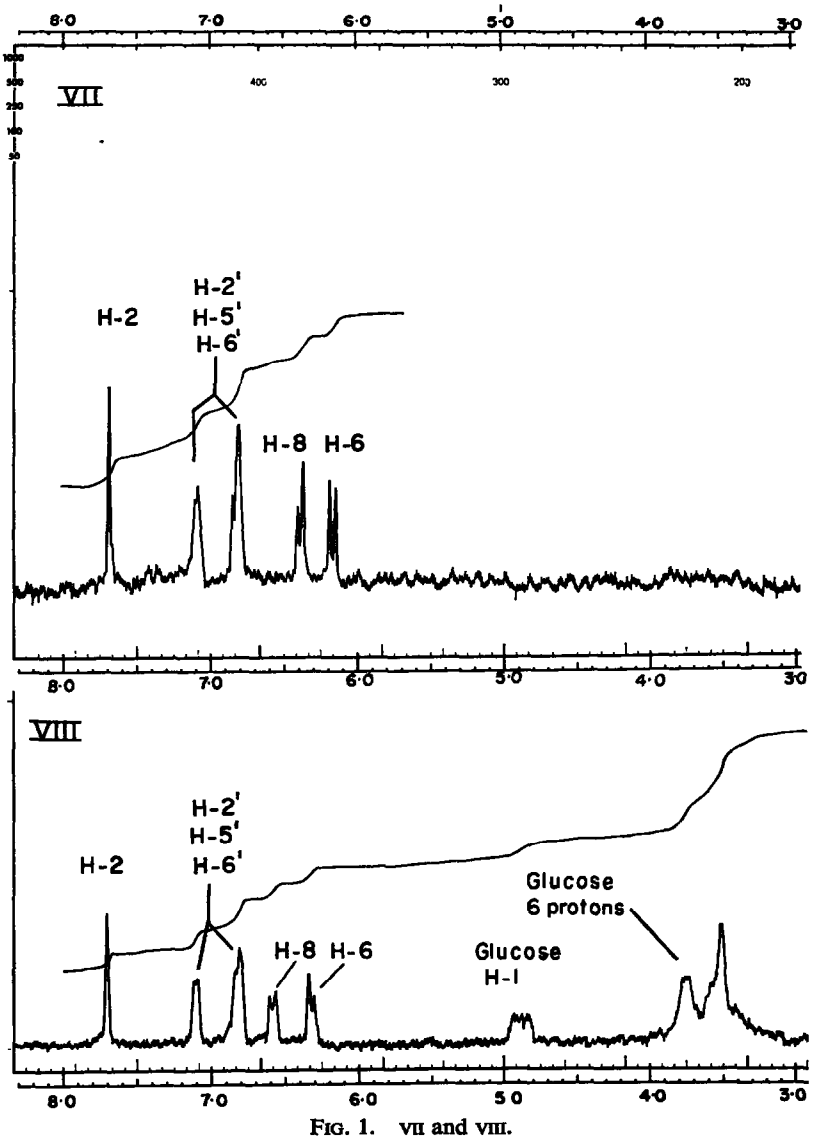


FIG. 1. VII and VIII.

TABLE 1. NMR PEAKS OF TRIMETHYLSILYL DERIVATIVES OF FLAVONOIDS AND COUMARINS*

	A-ring			B-ring		C-ring	Substituents
Flavonols							
Penduletin, 5,4′-dihydroxy-3,6,7-trimethoxy-flavone	H-8 6·46		H-2′ H-6′ 7·89d (8·5)	H-3′ H-5′ 6·82d (8·5)			OMe at 3, 6 and 7 3·7, 3·81, 3·88
Pendulin, penduletin-4′-glucoside	H-8 6·49		H-2′ H-6′ 7·98 (8·5)	H-3′ H-5′ 7·04 (8·5)			OMe at 3, 6 and 7 3·72, 3·84, 3·90 4′-glucose H-1, 4·92br 6 protons, 3·30–4·0
Hyperin, quercetin-3-galactoside	H-8 6·44d (2·5)	H-6 6·12d (2·5)	H-2′ 7·32d (2·5)	H-5′ 6·8d (8·5)	H-6′ 7·75q (8·5, 2·5)		3-galactose H-1, 5·61d(7) 6 protons, 3·3–4·0
Isoflavones							
Irigenin, 5,7,5′-trihydroxy-6,3′,4′-trimethoxy-isoflavone	H-8 6·42		H-2′ H-6′ 6·58d (2·5) 6·7d (2·5)			H-2 7·65	OMe at 6, 3′ and 4′ 3·7, 3·77, 3·80
Iridin, irigenin-7-glucoside	H-8 6·61		H-2′ H-6′ 6·61d (2·5) 6·71d (2·5)			H-2 7·71	OMe at 6, 3′ and 4′ two at 3·77, one at 3·84 7-glucose H-1, 4·98br 6 protons, 3·3–4·0
Irisolidone, 5,7-dihydroxy-6,4′-dimethoxy-isoflavone	H-8 6·44	H-2′ H-6′ 7·35d (8·5)	H-3′ H-5′ 6·82d (8·5)			H-2 7·63	OMe at 6 and 4′ 3·72, 3·79
Coumarins							
Aesculetin, 6,7-dihydroxy-coumarin		H-3 6·14d (9·5)	H-4 7·55d (9·5)	H-5 6·88	H-8 6·71		
Aesculin, aesculetin -6-glucoside		H-3 6·16d (9·5)	H-4 7·54d (9·5)	H-5 7·02	H-8 6·73		6-glucose H-1, 5·0br 6 protons, 3·1–3·8

* Values are given in ppm relative to tetramethylsilane as an internal reference. All signals correspond to the number of protons indicated. Singlets are unmarked, multiplets are described as follows: d, doublet, q, quarter, br, complex, broad signal. Numbers in parentheses denote coupling constants in c/s. All compounds were totally trimethylsilyated. The assignments for the H-5 and H-8 protons of the coumarins are tentative.

In contrast to glucose, the C-1 proton of rhamnose has an axial–equatorial coupling ($J \sim 2$ c/s). It gives a signal around 4·9 ppm when preesnt in a neohesperidoside as in naringin.[2] In the rhamnoglucosyl moiety of IV, the methyl group was discernible between 0·8

and 1·0 ppm, overlapping spinning sidebands of the trimethylsiloxyl groups (not recorded in Fig. 1). The rhamnose C-1 proton appears near 4·5 ppm, typical for rutinosides such as hesperidin[2]. As an equatorial–equatorial and axial–equatorial coupling of about 2 c/s can occur in the C-1 proton signal in selected conformations of α- and β-rhamnose, respectively, this method does not allow the determination of the nature of the rhamnose linkage, in contrast with the case of glucose.

In Table 1, NMR data are recorded for the trimethylsilyl derivatives of several flavonoids whose NMR spectra have not been previously reported. Among these are substances with a sterically hindered C-5 hydroxyl group, such as iridin, 7,5,3′-trihydroxy-6,4′,5′-trimethoxy isoflavone-7-glucoside. In addition, Table 1 includes the first NMR data for trimethylsilyl ethers of coumarins, aesculetin and its 6-glucoside.

The sugar components of the *Baptisia* glycosides were confirmed, after hydrolysis, by the elegant gas chromatographic procedure of Sweeley *et al.*[5] The isolation and further characterization of these and other *Baptisia* flavonoids will be described in a later publication.

EXPERIMENTAL

All the NMR spectra were recorded on a Varian A-60 spectrometer in carbon tetrachloride relative to internal tetramethylsilane. The trimethylsilyl ethers of the flavonoids were prepared by the procedure previously described.[2] Often the total conversion of flavonoids with sterically hindered hydroxyl groups to their trimethylsilyl ethers required a reaction time of more than 1 hr at room temperature.

The nature of the sugars in all the *Baptisia* glycosides was established by hydrolyzing the glycosides in 2 N HCl. The sugar was obtained after absorption of the aglycone over a nylon powder column, packed in water. The anomeric mixture of the sugar was then trimethylsilylated and analyzed by gas chromatography as recommended by Sweeley *et al.*[5] The retention times of the peaks were compared with those of authentic standards.

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[5a] C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, *J. Am. Chem. Soc.* **85**, 2497 (1963).
[5b] J. Kagan and T. J. Mabry, *Anal. Chem.* In press (1965).