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(−)-5′-METHOXYSATIVAN, A NEW ISOFLAVAN FROM ALFALFA

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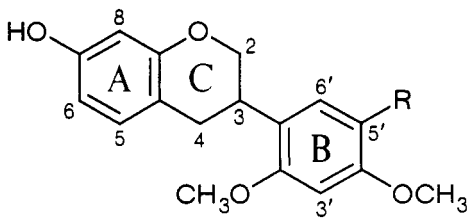
ABSTRACT.—(−)-5′-Methoxysativan, 7-hydroxy-2′,4′,5′-trimethoxyisoflavan was isolated from alfalfa (*Medicago sativa*) foliage and characterized by its ^{1}H nmr and mass spectra. Assignment of the chemical shifts for 5′-methoxysativan allowed definitive interpretation of the spectrum of sativan (7-hydroxy-2′,4′-dimethoxyisoflavan).

Recently, it was reported that alfalfa foliage contains an uncharacterized methoxy analogue of sativan (7-hydroxy-2′,4′-dimethoxyisoflavan) (1). This compound has now been identified from mass and nmr spectra as 5′-methoxysativan (7-hydroxy-2′,4′,5′-trimethoxyisoflavan) [**1**].

Four compounds reported previously (1) have been isolated here from a different sample of alfalfa foliage. The isoflavan, 5′-methoxysativan, eluted very close to the two pterocarpans, medicarpin (3-hydroxy-9-methoxypterocarpan) and 4-methoxymedicarpin (3-hydroxy-4,9-dimethoxypterocarpan), making separation by hplc difficult. Successful separation was achieved by using MeOH-H_2O (1:1) in an isocratic mode, and enough sample was obtained for characterization by ms and ^{1}H nmr.

Three methoxyl groups were apparent in the ^{1}H-nmr spectrum of **1** (see Table 1), and a major ion at *m/z* 194 in the ms for the retro-Diels-Alder fragment indicated that the methoxyl groups were on ring B. The two protons of ring B did not show an observable coupling in ^{1}H nmr and, therefore, were suspected to be para to each other. Of the six different possible arrangements of the three methoxyl groups on ring B, only one structure, the 2,4,5-trimethoxyphenyl moiety, has the unsubstituted positions para to each other. There are several examples in the literature of this 2′,4′,5′ substitution of isoflavans, but all contain methylenedioxy in the 4′,5′ positions (2). ^{1}H-nmr data for astraciceran, 2′-*O*-methylleiocin, and 2′-*O*-methylisoleiocin (3) support our assignments. The three aromatic protons of ring A have ortho, ortho/meta, and meta couplings in the ^{1}H nmr. Therefore, only two arrangements are possible: the hydroxyl group is at 6 or 7, and regardless of which position is correct, the shift of H-5 is the farthest downfield of the protons on ring A. The aromatic proton at δ 6.93 (the farthest downfield shift) is ortho-coupled to that at δ 6.37, which is meta-coupled to that at δ 6.34 (Table 1). This arrangement eliminates placement of the hydroxyl group at position 6 and places it on 7 (4). The ms, ^{1}H-nmr, and optical rotation data characterize this methoxy sativan as (−)-5′-methoxysativan.



1 R=OMe
2 R=H

[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

TABLE 1. ^{1}H-nmr Data for 5′-Methoxysativan [**1**] and Sativan [**2**].[a]

Proton	5′-Methoxysativan [**1**]	Sativan [**2**]
2ax	4.01 t J = 10.1, 10.1	3.98 t J = 10.0, 10.0
2eq	4.28 ddd J = 10.1, 3.5, 1.8	4.28 ddd J = 10.0, 3.4, 2.0
3	3.57 m	3.55 m
4ax	2.97 dd J = 15.5, 10.2	2.95 dd J = 15.5, 10.2
4eq	2.86 ddd J = 15.5, 5.5, 1.6	2.86 ddd J = 15.5, 5.4, 1.8
5	6.93 d J = 8.0	6.92 d J = 8.0
6	6.37 dd J = 8.0, 2.5	6.37 dd J = 8.0, 2.5
8	6.34 d J = 2.5	6.34 d J = 2.5
3′	6.54 s	6.48 d J = 2.5
5′		6.45 dd J = 8.1, 2.5
6′	6.68 s	7.01 d J = 8.1
OH	4.58 br s	4.55 br s
OMe	3.78 s	3.79 s
	3.81 s	3.80 s
	3.88 s	

[a]300 MHz (delta scale, coupling constants in Hz, $CDCl_3$).

Assignments of the aromatic protons for 5′-methoxysativan have now made it possible to assign those of sativan [**2**]. Both aromatic rings (the A and B rings) of sativan have 1,2,4 substitution that produces similar coupling patterns and, therefore, definitive assignments have usually not been made (5). Comparison of the structures **1** and **2** shows that the fused rings, A and C, are the same for both compounds and should have the same chemical shifts for the protons involved. Examination of Table 1 reveals that all of the shifts for both **1** and **2** are nearly the same (except for the protons of ring B). The missing proton signal of **1** (that has been replaced with a methoxyl group) is in ring B. Thus, the farthest downfield shift is assigned to H-6′ (in **2**) and not to H-5 where it has been placed by some authors for similarly substituted isoflavans, for example, vestitol (6,7).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured with a Perkin-Elmer 241 polarimeter. ^{1}H nmr were obtained at 300 MHz with $CDCl_3$ as the solvent. Ms were obtained at 70 eV in the electron impact mode. Both gc and gc-ms were obtained with a 15-m DB-1 column, temperature-programmed at 4°/min from 100° to 300°. A 6-cm (i.d.) × 62-cm glass column, slurry-packed with 500 g 60–200 mesh Si gel (J.T. Baker), was used for cc. Commercial 20 × 20 × 0.25 cm precoated Si gel 60 F_{254} (Merck) plates were used for tlc. A 9.4 × 250 mm Zorbax ODS column (DuPont) and refractive index and uv (254 nm) detectors were used for hplc.

PLANT MATERIAL.—Alfalfa (*Medicago sativa* L. "CUFF," Leguminosae) foliage was provided by Michigan State University, East Lansing, MI, from a planting of a known cultivar.

EXTRACTION.—The ground sample (3970 g) was extracted in a percolator with 95% EtOH. Solvent was removed under vacuum in a rotating evaporator. The residue (345 g) was dissolved in H_2O-MeOH (1:1) and extracted with *n*-hexane. To the aqueous solution was added more H_2O making it H_2O-MeOH (3:1). This solution was extracted with EtOAc. The EtOAc extract (21 g) was chromatographed on a Si gel column with step-gradient elution, beginning with 2.5 liters EtOAc-hexane (1:4), followed by 2 liters each EtOAc-hexane (4:1), EtOAc, EtOAc-MeOH (1:1), and MeOH. The column fractions (500 ml each, except for the first two, which were 1 liter each) were monitored by tlc with EtOAc-MeOH (20:1) as the developing solvent. Spraying the air-dried developed plate with chromate/H_2SO_4 produced a pink color with medicarpin (before charring the plate). Only column fraction 5 contained a spot by tlc showing the pink color.

Results from gc of column fractions 4, 5, and 6 suggested that the major peaks of each fraction were sativan, medicarpin, and 4-methoxymedicarpin, respectively. Preparative hplc, with MeCN-H_2O (1:1) at 4 ml/min, separated column fraction 6 (1 g) into 4-methoxymedicarpin and fraction 6-B. Fraction 6-B was separated into fraction 6-B-1 and medicarpin by use of MeCN-EtOH-

H_2O (1:1:2) at 4 ml/min. The solvent was changed to MeOH-H_2O (55:45) and the flow rate to 5 ml/min in order to separate fraction 6-B-1 into 4-methoxymedicarpin and impure methoxysativan. Chromatographically pure methoxysativan (10 mg; 0.00025% yield) was obtained by hplc with MeOH-H_2O (1:1) at 5 ml/min.

COMPOUND IDENTIFICATION.—Preliminary structural identification was established by gc-ms, and confirmatory evidence was afforded by ^{1}H nmr.

5′-Methoxysativan [**1**].—$[\alpha]^{25}D$ −12.7° (*c* = 2.7 mg/ml, MeOH); *m/z* (rel. int.) 317 (18), $[M]^+$ 316 (100), 281 (12), 270 (15), 221 (14), 194 (67), 182 (56), 181 (32), 179 (62), 167 (13), 166 (19), 164 (16), 151 (18), 148 (14), 147 (22), 136 (10), 135 (12), 121 (18), 107 (11), 91 (15), 77 (18), 65 (15), 40 (40); ^{1}H nmr see Table 1.

Sativan [**2**].—$[\alpha]^{24}D$ −9.9° (*c* = 3.3 mg/ml, MeOH); *m/z* (rel. int.) 287 (18), $[M]^+$ 286 (100), 165 (11), 164 (75), 152 (34), 151 (71), 149 (59), 147 (10), 135 (9), 121 (45), 107 (7), 91 (16), 77 (17), 65 (9), 40 (19); ^{1}H nmr see Table 1. For literature values of nmr and ms see Braz Filho *et al.* (5) and Ingham (8).

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LITERATURE CITED

1. R.W. Miller, R. Kleiman, R.G. Powell, and A.R. Putnam, *J. Nat. Prod.*, **51**, 328 (1988).
2. J.L. Ingham, in: "Chemistry of Organic Natural Compounds." Ed. by W. Herz, H. Grisebach, and G.W. Kirby, Springer-Verlag, Wien, 1983, Vol. 43, pp. 1–266.
3. F.R. van Heerden, E.V. Brandt, and D.G. Roux, *J. Chem. Soc., Perkin Trans. 1, 137 (1978).*
4. *A. Pelter and P.I. Amenechi, J. Chem. Soc., Sect. C,* 887 (1969).
5. R. Braz Filho, O.R. Gottlieb, A.P. Mourao, A.I. DaRocha, and F.S. Oliveira, *Phytochemistry*, **14**, 1454 (1975).
6. O.R. Gottlieb, A.B. de Oliveira, T.M.M. Goncalves, G.G. de Oliveira, and S.A. Pereira, *Phytochemistry*, **14**, 2495 (1975).
7. K. Kurosawa, W.D. Ollis, B.T. Redman, I.O. Sutherland, and O.R. Gottlieb, *Phytochemistry*, **17**, 1413 (1978).
8. J.L. Ingham, *Phytochemistry*, **16**, 1279 (1977).

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