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A PTEROCARPAN AND TWO ISOFLAVANS FROM ALFALFA

GAYLAND F. SPENCER,* BARRY E. JONES, RONALD D. PLATTNER, DAVID E. BARNEKOW,†
LINDA S. BRINEN‡ and JON CLARDY‡

U.S. Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL 61604, U.S.A.; ‡Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, NY 14853, U.S.A.

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Key Word Index—*Medicago sativa*; Leguminosae; lucerne; 10-methoxymedicarpin; 7-hydroxy-2',3',4'-trimethoxyisoflavan; 7,5'-dihydroxy-2',3',4'-trimethoxyisoflavan; 3'-methoxysativan; 3'-methoxy-5'-hydroxysativan; 3-hydroxy-9,10-dimethoxypterocarpan.

Abstract—(−)-6*aR*,11*aR*-Dihydro-3-hydroxy-9,10-dimethoxy-6*H*-benzofuro[3,2*c*] [1]-benzopyran (10-methoxymedicarpin), (+)-(2,3,4,-trimethoxyphenyl)-2,3-dihydro-7-hydroxy-4*H*-1-benzopyran (7-hydroxy-2',3',4'-trimethoxyisoflavan) and (+)-(2,3,4-trimethoxy-5-hydroxyphenyl)-2,3-dihydro-7-hydroxy-4*H*-1-benzopyran (7,5'-dihydroxy-2',3',4'-trimethoxyisoflavan) were isolated for the first time from dried *Medicago sativa* hay. Structural assignments were based on ^{1}H NMR and mass spectra, X-ray crystallography, and optical rotations.

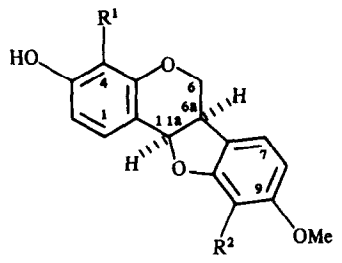
INTRODUCTION

Medicarpin (**1a**), 4-methoxy medicarpin (**1c**), sativan (**2a**) and 5'-methoxysativan (**2b**) are produced by alfalfa (*Medicago sativa* L.) [1–3] and are under investigation as plant growth regulators at this laboratory [4]. In the process of extracting and purifying gram quantities of these compounds from large batches of alfalfa hay, we have isolated minor amounts of the previously unknown compounds **1b**, **2c** and **d**.

RESULTS AND DISCUSSION

Compound **1b** did not crystallize but gave a single spot by TLC and a single peak by GC-MS and HPLC. The mass spectrum was virtually identical to that of 4-methoxymedicarpin (**1c**) [2] but the NMR spectrum, although similar to that of **1c** in shifts and multiplicities, was clearly that of a different compound. Resonances for the methoxyl groups were shifted slightly downfield (δ3.75, 3.90 for **1c** [1] vs δ3.83, 3.92 for **1b**) and the aromatic protons exhibited a similar trend. Shifts for the other five protons also did not exactly coincide with those of **1c** but did indicate the presence of a pterocarpan ring system [5]. The resonances of the aromatic protons revealed the familiar ABC pattern for one of the rings (δ6.41, 6.54 and 7.43) and two doublets indicative of *ortho* substitution for the other (δ6.45 and 6.87, J=8.4 Hz). Preston [6] showed that reduction of a pterocarpan over Pd/C yields an isoflavan that, under mass spectral analysis, undergoes retro-Diels–Alder (RDA) fragmentation to intense ions that locate the positions of substitution. The RDA ion at m/z 180 proved that the isoflavan produced from **1b** has two methoxyl groups on the phenyl ring and, because the NMR spectrum located the two ring protons *ortho* to each other, the methoxyl groups must be either at C-7 and C-8 or C-9 and C-10. Placement of the methoxyl groups at positions 9 and 10 is supported

*Author to whom correspondence should be addressed.
†Present address: DowElanco, Building 9001, P.O. Box 1706, Midland, MI 48641, U.S.A.



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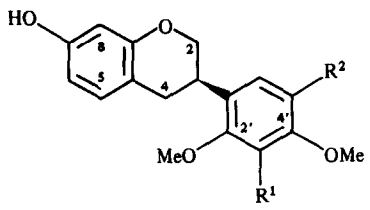

2

	R^1	R^2
a	H	H
b	H	OMe
c	OMe	H
d	OMe	OH

not only on biogenetic grounds (all known pterocarpans are substituted at C-9), but also because the shift at δ6.87 is more easily attributed to a C-7 proton than one at C-9. Finally, the absolute configuration of **1b** can be assigned from its rotation because all *cis*-pterocarpans with negative rotation possess the 6*aR*; 11*aR* configuration [7, 8].

The NMR and mass spectra of **2c** confirm an isoflavan with three methoxyl groups on the phenyl ring and that the remaining protons are *ortho* to each other (RDA at *m/z* 194; δ6.63 and 6.78, J=8.6 Hz) as is shown in the computer-generated perspective drawing in Fig. 1 obtained by X-ray crystallography. Its conformation is given from the magnitude of the couplings of H-2, H-3 and H-4 protons that place the phenyl ring in the equatorial position [9]. Likewise, the structure of **2d** was deduced from spectral and crystallographic data. A computer generated drawing of **2d** is shown in Fig. 2. The ^{1}H NMR signal for the axial proton on C-2 was obscured by the methoxyl signals, but the remaining signals serve to identify the conformation.

Considering the lengthy steps needed to isolate and purify the above compounds and the concomitant losses of material, it is difficult to accurately determine their abundance in the alfalfa hay. We estimate that they are present in amounts no greater than 20 ppm.

EXPERIMENTAL

HPLC, GC-MS and NMR equipment has been described previously [1, 3, 10, 11]. Mp: uncorr. Optical rotations were measured in $CHCl_3$.

Alfalfa was harvested from a 6- to 8-year-old plantation near Peoria, IL. A voucher specimen is on deposit in the herbarium at the University of Illinois, Urbana, IL. Field-dried hay was chopped into 0.5 cm lengths and 20 kg batches were soaked in 95% EtOH for 3 days. The extract was drained and concd to a viscous liquid by evapn at red. pres. About 1 l of this concentrate was added to 1.5 l of 1 M HCl and then extracted ×4 with 600 ml aliquots of CH_2Cl_2. The combined extracts were evapd to near dryness (*ca* 100 g) and then applied to a 16 × 38 cm

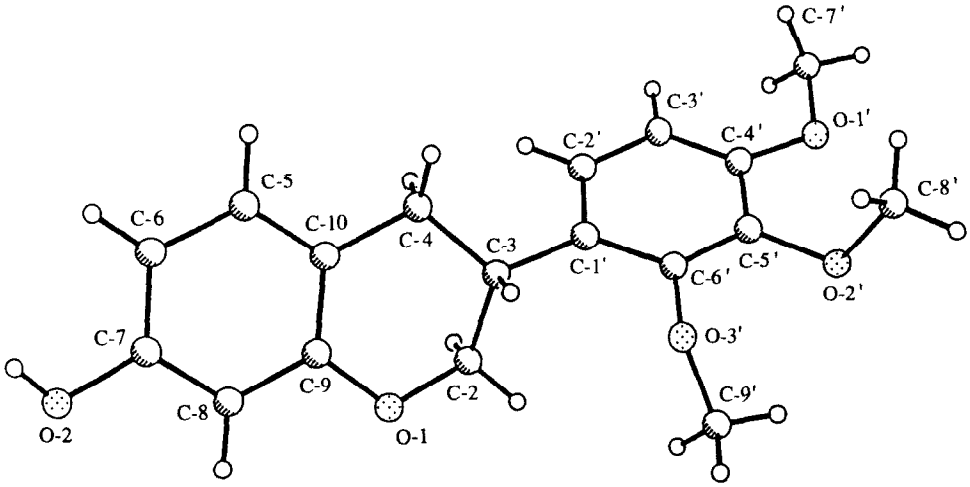

Fig. 1. Computer generated perspective drawing of **2c** from X-ray crystallography.

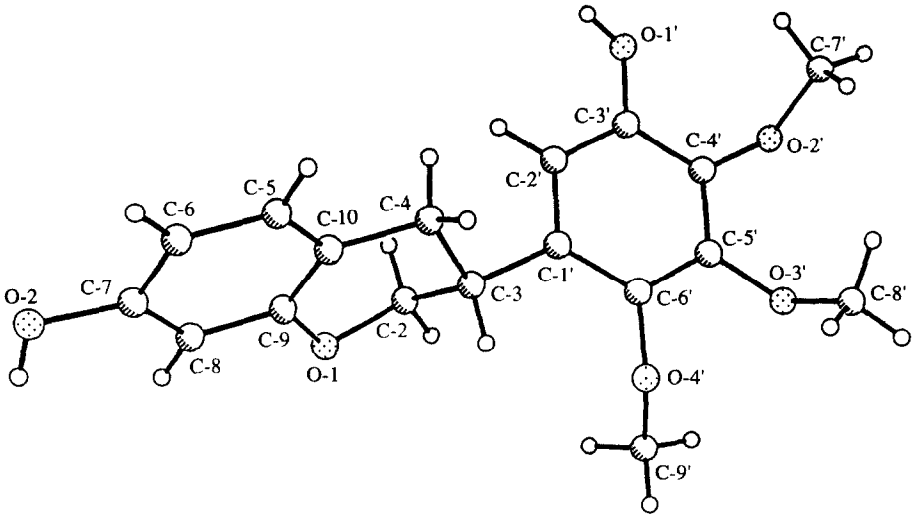

Fig. 2. Computer generated perspective drawing of **2d** from X-ray crystallography.

Sephadex LH-20 (Pharmacia) column and eluted with CH_2Cl_2–MeOH (1:1). Each 2 l of eluate was evapd and monitored for pterocarpan and isoflavan content by TLC on silica with $CHCl_3$–MeOH (19:1) as the solvent system. Fractions of interest were further separated by HPLC at a flow rate of 70 ml min^{-1} through a 47 × 300 cm reverse-phase C_{18} column (particle size 105 μ) with a linear gradient from H_2O–MeCN (24:1) to (11:9) over 35 min. The effluent was monitored by UV at 310 nm. Final purification by HPLC was accomplished with a 9.4 × 250 mm Zorbax C_{18} (DuPont) column with an isocratic solvent system of MeCN–H_2O (1:1) at 4 ml min^{-1} monitored by a differential refractometer, followed by prep. TLC on silica with CH_2Cl_2–MeOH (49:1) as the development solvent.

(−)-6aR,11aR-*Dihydro*-3-*hydroxy*-9,10-*dimethoxy*-6H-*benzofuro*-[3,2c] [1]-*benzopyran* (**1b**). $[\alpha]_D^{27}$ −107° ($CHCl_3$; *c* 0.045) EIMS, 70 eV, *m/z* (rel. int.) 301 (20), 300 (100, $[M]^+$), 285 (30, $[M-Me]^+$), 269 (10), 253 (5), 167 (5), 147 (10). ^{1}H NMR (300 MHz, $CDCl_3$), δ3.52 (*ddd*, *J* = 10.9, 6.7, 4.9 Hz, H-6a), 3.67 (*dd*, *J* = 10.9, 10.9 Hz, H-6_{ax}), 3.83 (*s*, OMe), 3.92 (*s*, OMe), 4.23 (*dd*, *J* = 10.9, 4.9 Hz, H-6_{eq}), 5.2 (*br s*, OH), 5.51 (*d*, *J* = 6.7 Hz, H-11a), 6.41 (*d*, *J* = 2.5 Hz, H-4), 6.45 (*d*, *J* = 8.1 Hz, H-8), 6.54 (*dd*, *J* = 8.4, 2.5 Hz, H-2), 6.87 (*d*, *J* = 8.1 Hz, H-7), 7.43 (*d*, *J* = 8.4 Hz, H-1).

Hydrogenation of compound **1b**. A 2 mg sample of **1b** was dissolved in 2 ml EtOH to which 10% Pd/C was added, and H_2 bubbled through the mixture for 2 hr. GC-MS gave two peaks of nearly equal intensity that were identified as **1b** and the hydrogenation product: EIMS, 70 eV, *m/z* (rel. int.) 303 (20), 302 (100, $[M]^+$), 180 (95, $[RDA]^+$), 168 (66), 167 (50), 133 (20), 77 (10), 66 (10).

(+)-(2,3,4-*trimethoxyphenyl*)-2,3-*Dihydro*-7-*hydroxy*-4H-1-*benzopyran* (**2c**). $[\alpha]_D^{27}$ +6.5° ($CHCl_3$; *c* 1.150), mp 168–170° (uncorr.); EIMS, 70 eV, *m/z* (rel. int.) 317 (10), 316 (55, $[M]^+$), 194 (95, $[RDA]^+$), 182 (80), 181 (50), 179 (100, $[RDA-Me]^+$), 166 (35), 151 (44), 147 (18), 136 (15), 121 (14), 107 (17), 91 (25). ^{1}H NMR (300 MHz, $CDCl_3$) 2.87 (*ddd*, *J* = 9.8, 6.4, 2.5 Hz, H-4_{eq}), 2.91 (*dd*, *J* = 9.8, 3.5 Hz, H-4_{ax}), 3.53 (*m*, H-3), 3.83 (*s*, OMe), 3.87 (*s*, OMe), 3.89 (*s*, OMe), 3.98, (*dd*, *J* = 10.2, 10.2 Hz, H-2_{ax}), 4.26 (*ddd*, *J* = 10.3, 3.5, 1.9 Hz, H-2_{eq}), 5.35 (*br s*, OH), 6.35 (*d*, *J* = 2.5 Hz, H-8), 6.38 (*dd*, *J* = 8.0, 2.5 Hz, H-6), 6.63 (*d*, *J* = 8.6 Hz, H-5'), 6.78 (*d*, *J* = 8.6 Hz. H-6'), 6.92 (*d*, *J* = 8.0 Hz, H-5).

(+)-(2,3,4-*trimethoxy*-5-*hydroxyphenyl*)-2,3-*Dihydro*-7-*hydroxy*-4H-1-*benzopyran* (**2d**). $[\alpha]_D^{27}$ +7.5° ($CHCl_3$; *c* 0.480), mp 137–138° (uncorr.); EIMS, 70 eV, *m/z* (rel. int.) 333 (18), 332 (97, $[M]^+$), 210 (100, $[RDA]^+$), 198 (33), 195 (50, $[RDA-Me]^+$), 183 (30), 163 (44), 147 (15), 135 (32), 122 (22), 107 (20), 94 (27). ^{1}H NMR (300 MHz, $CDCl_3$) δ2.84 (*ddd*, *J* = 11.7, 6.5, 2.6 Hz, H-4_{eq}), 2.88 (*dd*, *J* = 11.7, 2.9 Hz, H-4_{ax}), 3.54 (*m*, H-3), 3.80 (*s*, OMe), 3.93 (*s*, OMe), 3.94 (*s*, OMe), 3.94 (*dd*, *J* = 10.0, 10.3 Hz, H-2_{ax}), 4.25 (*ddd*, *J* = 10.3, 2.6, 2.0 Hz, H-2_{eq}), 4.30 (*s*, OH), 5.50 (*s*, OH), 6.35 (*d*, *J* = 2.6 Hz, H-8), 6.38 (*dd*, *J* = 8.0, 2.6 Hz, H-6), 6.45 (*s*, H-6'), 6.92 (*d*, *J* = 8.0 Hz, H-5).

Single crystal X-ray diffraction analysis of (+)-(2,3,4-*trimethoxyphenyl*)-2,3-*dihydro*-7-*hydroxy*-4H-1-*benzopyran* (**2c**). A rod-like crystal with dimensions of 0.20 × 0.30 × 0.60 mm grown from acetone was selected for X-ray measurements. Preliminary X-ray photographs displayed triclinic symmetry and accurate lattice constants of *a* = 8.550 (4), *b* = 10.169 (8), *c* = 10.380 (6) Å and α = 65.67 (5), β = 76.87 (4), γ = 88.06 (5)° were determined from least-squares analysis of 30 diffractometer-measured 2θ values. All diffraction maxima with 2θ ≤ 115° were collected with a computer controlled four-circle diffractometer using θ:2θ scans and graphite monochromated Cu-K_α (1.54718 Å) radiation. A total of 2351 unique reflections were measured this way and after correction for Lorentz, polarization and background effects, 2191 (93%) were judged observed $|F_o| \geq 5\sigma|F_o|$. Two molecules of **2c** were found to form the asymmetric unit in the space group P1 and a phasing model was found routinely using direct methods. Full-matrix least squares refinements with anisotropic heavy atoms and isotropic riding hydrogen atoms converged to a final crystallographic discrepancy index of 5.14%. A computer generated perspective drawing of **2c** is given in Fig. 1. Archival crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, Cambridge, U.K.

Single crystal X-ray diffraction analysis of (+)-(2,3,4-*trimethoxy*-5-*hydroxyphenyl*)-2,3-*dihydro*-7-*hydroxy*-4H-1-*benzopyran* (**2d**). A 0.1 × 0.1 × 0.7 mm flat plate of **2d** from CH_2Cl_2 and isooctane crystallized in the orthorhombic space group $P2_12_12_1$ with *a* = 5.5780 (1), *b* = 13.401 (3), *c* = 22.744 (4) Å. A total of 1378 unique diffraction maxima were collected at room temp. using Cu-K_α (1.54718 Å) radiation θ:2θ scans. After correction for Lorentz, polarization and background effects, 1108 (80%) were judged observed $|F_o| \geq 4\sigma|F_o|$. A solution from routine application of direct methods and full-matrix least-squares refinements with anisotropic heavy atoms and isotropic riding hydrogen atoms yielded a crystallographic R-factor of 4.60%. A computer generated perspective drawing of **2d** is given in Fig. 2. Archival crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, Cambridge, U.K.

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