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IPOMOPSIN, A NEW BISCOUMARIN FROM *IPOMOPSIS AGGREGATA*

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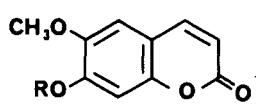
ABSTRACT.—Fractionation of an aqueous MeOH extract of *Ipomopsis aggregata* afforded a new biscoumarin, ipomopsin (**4**), and the widely distributed coumarin, scopoletin (**1**). The structure of **4** was established through the interpretation of spectral data. ^{1}H-nmr spectral data for the biscoumarin matsukaze-lactone (**6**) are reported for the first time.

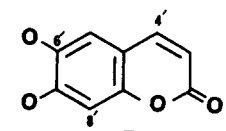
We have previously reported that cucurbitacin B, isocucurbitacin B, and 3-epi-isocucurbitacin B were responsible for the cytotoxic activity of an aqueous MeOH extract of *Ipomopsis aggregata* (Pursh) V. Grant (Polemoniaceae) (1). As a continuation of studies on this plant, we present evidence for the structure of a new biscoumarin, ipomopsin, and report on the isolation of the widely distributed coumarin, scopoletin (**1**).

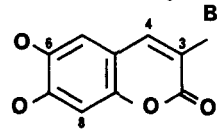
The uv spectrum of ipomopsin was very similar to that of **1**, and the ir spectrum displayed absorptions in the hydroxyl (ν max 3340 cm^{-1}) and carbonyl regions (1700 cm^{-1}). A molecular ion was observed at *m/z* 382 with significant fragment ions at *m/z* 367 (M^+-15), *m/z* 354 (M^+-28), *m/z* 339 (M^+-28-15), and *m/z* 336 (M^+-28-18), typical of those of coumarins (2). The ^{1}H-nmr spectrum of ipomopsin in DMSO-d_6 at 60 MHz showed a pair of doublets (J=9.2 Hz) at δ 6.24 and 7.97, which could be assigned to H-3′ and H-4′ on a coumarin nucleus, **A**, and a singlet at δ 7.90 attributable to H-4 of a 3-substituted coumarin nucleus, **B** (3). Two aromatic methoxy groups were observed at δ 3.84 and 3.90 together with three singlets at δ 6.88, 7.26, and 7.32. These data indicated that ipomopsin was a biscoumarin in which C-3 of one unit was attached to C-5 or C-8 of a second unit. Because the orientation of the hydroxy/methoxy groups on the nuclei also required resolution, a total of 12 possible structures could be proposed for ipomopsin as shown in Table 1.[2]



1 R=H

3 R=Ac





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[2]A preliminary idea of the possible structure of ipomopsin was gained through comparison of the chemical shifts induced in the aromatic protons on acetylation of scopoletin and ipomopsin as shown in Table 2. Thus, in scopoletin acetate (**3**), the proton *ortho* to the acyl group is shifted downfield, whereas the *meta* proton is shifted upfield an almost equivalent amount. In ipomopsin diacetate, two ArH were shifted upfield and only one downfield, thereby eliminating structures **2a, 2b** and **2g-2l.** In addition, the downfield shift of the 3-H on acetylation of the 7-hydroxy group of scopoletin is matched by a shift of the 3′-H in ipomopsin, which may eliminate **2c** and **2d**, leaving a distinction to be made between **2e** and **2f**. The uv spectra of scopoletin and ipomopsin are quite similar, and on the addition of base, a quite analogous bathochromic shift 47 nm shift is observed for the long wavelength band in both instances. This favors structure **2e** over structure **2f**.

TABLE 1. Distinction of the Isomers of Ipomopsin

Compound	Position of OCH_3		Linkage point of A-3 in B unit	No. H adj. OCH_3	No. ^5J 4-H	Ar-H Decoupled *and* showing nOe[a]
	A Unit	B Unit				
2a	6	6′	5′	1	2	2 different
2b	7	6′	5′	1	2	1 same 1 different
2c	6	7′	5′	2	2	1 same 1 different
2d	7	7′	5′	2	2	2 same
2e	6	6′	8′	2	1	different
2f	7	6′	8′	2	1	same
2g	6	7′	8′	1	1	different
2h	7	7′	8′	1	1	same
2i	6,7	—	5′	2	2	1 different
2j	6,7	—	8′	2	1	same
2k	—	6′,7′	5′	1	2	1 same 1 different
2l	—	6′,7′	8′	1	1	different

[a]"Same" indicates that the ArH giving a nOe on irradiation of a OCH_3 group is the same as the ArH long-range coupled to the 4-H, *i.e.*, the OCH_3 is at C-7 with a proton at C-8. "Different" indicates that the ArH giving a nOe on irradiation of a OCH_3 group is different than the ArH which is long-range coupled to the 4-H, *i.e.*, the OCH_3 is at C-6 with a proton at C-8.

These structures could be distinguished through two experiments, one based on the anticipated nOe effect between an aromatic methoxy group and an adjacent proton, and the second, the long-range coupling between H-4 and H-8 observed in coumarins where these positions are unsubstituted (3). The rationale behind these experiments is shown in Table 1. Irradiation of the $ArOCH_3$ region indicates the number of adjacent ArH, and the number of long-range coupled C-8 protons may theoretically be deduced by irradiating the region of δ 7.95, which contains H-4 and H-4′. Rationalization of the data reduces the possible choices to two. A final distinction can then be made on whether or not the ArH showing a nOe are the same as those displaying long-range coupling. In order to minimize solubility problems and enhance the sensitivity and field dispersion, subsequent work was performed on the diacetate derivative at 360 MHz using $CDCl_3$ as the solvent (Figure 1).

Irradiation of the aromatic methoxy region at δ 3.880 caused a nOe effect of 16% at δ 7.008, and an equivalent nOe was observed at δ 7.058 when the methoxy group at δ 3.903 was irradiated. These results eliminate structures **2a, 2b, 2g, 2h, 2k,** and **2p.** The spectrum also indicates that there is long-range coupling (J=0.4 Hz) between H-4 and H-8 (δ 7.155) in only one of the nuclei.

More complete interspatial relationships were established through a two-dimensional nOe experiment (4) (Figure 2)[3] using $CDCl_3/C_6D_6$ as the solvent.[4] In this way, it was demonstrated that H-5′ exhibited nOe effects with H-4′ and a 6′-OCH_3, and that H-5 was spatially proximate to H-4 and a 6-OCH_3. This eliminates structures **2c, 2d, 2i,** and **2j,** leaving **2e** (now indicated as **4**) as the structure of ipomopsin. Confirmation of this was obtained through a COSY experiment (4) in which a pulse delay of 250 msec

[3]We thank Dr. Ralph E. Hurd, Nicolet Magnetics Corporation, Fremont, CA for conducting these experiments.

[4]C_6D_6 was added to enhance the chemical shift difference between H-4′ and H-4. Several other chemical shifts were also altered in this process: δ 2.349 (-OAc), 2.414 (-OAc), 3.865 (6-OCH_3), 3.910 (6′-OCH_3), 6.481 (H-3′), 6.958 (H-5′), 6.987 (H-5), 7.329 (H-8), 7.460 (H-4′) and 7.752 (H-4).

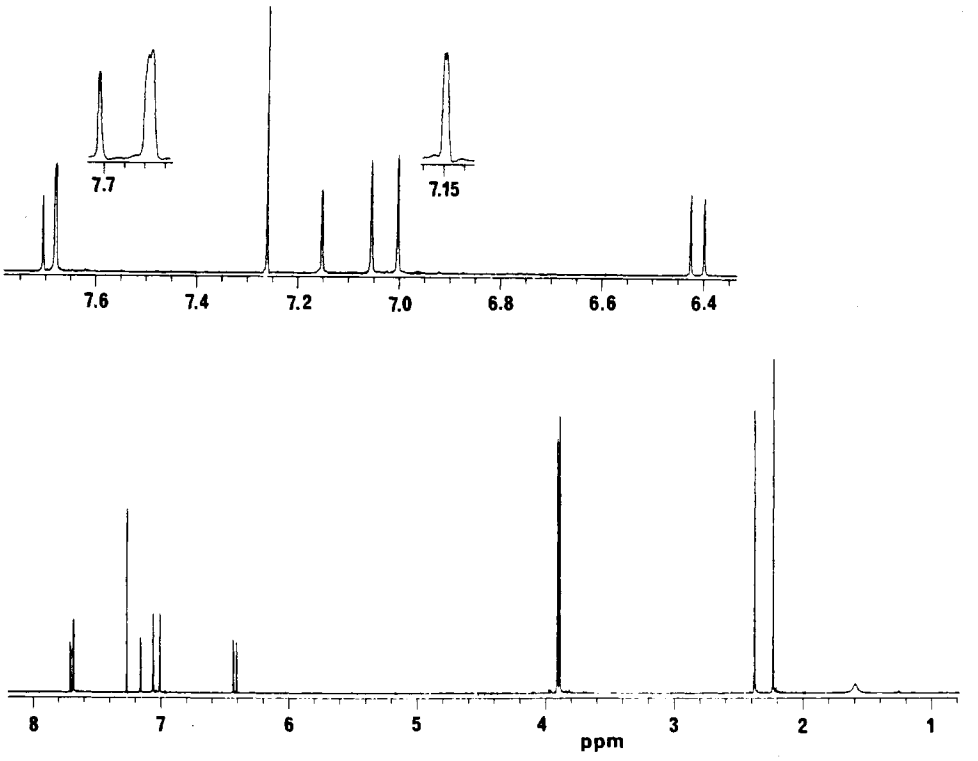


FIGURE 1. 360 MHz ^{1}H-nmr spectrum of ipomopsin diacetate in $CDCl_3$.

was used to enhance the long-range coupling in the molecule (Figure 3). It was thus established that only one of four-protons was coupled to an H-8, as expected for **4**.

Aryl-aryl linked biscoumarins are a rare group of natural products, the principal known examples of established structure being euphorbetin (5) and isoeuphorbetin (6) from *Euphorbia lathyris* L. (Euphorbiaceae), matusukaze-lactone from *Boenninghausenia japonica* (Sieb.) Nakai (Rutaceae) (7-9), bicoumol from *Ruta* sp. Tene. 29662 (10), and eriocephaloside from *Lasiosiphon eriocephalus* Decne. (Thymelaeaceae) (11). In addition, there have been several reports of bicoumarins formed through the coupling of coumarins with various oxidizing agents (12-15). Ipomopsin (**4**), therefore, is the first member of a new series of biscoumarins in which the pyran-2-one carbon atoms are joined to the second aryl unit.

In the course of these studies on ipomopsin (**4**), we also had the opportunity to examine the ^{1}H-nmr spectrum of matsukaze-lactone (**6**), which hitherto has not been described. Oxidative degradation had been used previously (7,8) to establish the location of the methoxy groups with respect to the linkage points between the two coumarin nuclei. Partial synthesis of the appropriately substituted biphenyl derivative allowed structure **6** to be selected over the alternative structures in which either the linkage was from C-8 to C-7′ (OCH_3 at C-6′) or was from C-8 to C-8′.

The ^{1}H-nmr spectrum at 360 MHz confirmed the structure of matsukaze lactone to be **6**, but some additional experiments were needed to assign unequivocally all of the proton resonances. Two methoxy resonances were observed at δ 3.816 and 3.844, and in the aromatic region, three pairs of doublets and a pair of singlets were observed. The latter, at δ 7.325 and 6.942, could be ascribed to H-5′ and H-8′, respectively, and could eliminate the possibility of a C-8-C-8′ linkage. Two deshielded doublets at δ 7.692 and 7.649 were assigned to H-4′ and H-4, and the two most shielded doublets at

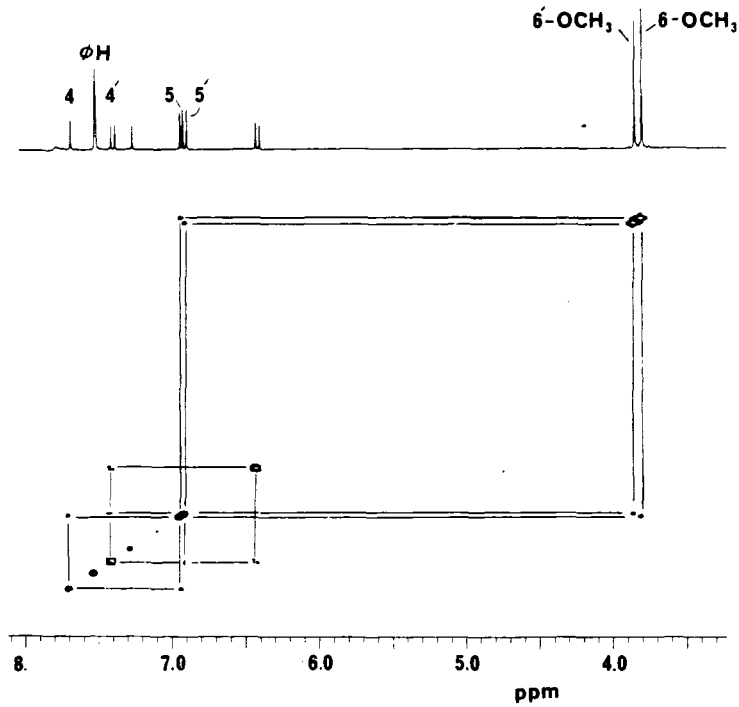


FIGURE 2. 2D nOe Experiment on ipomopsin diacetate in $CDCl_3$-C_6D_6 at 360 MHz.

δ 6.279 and 6.255 to H-3′ and H-3 (3). Any linkages involving C-3, C-3′, C-4 or C-4′ are, therefore, eliminated.

The remaining doublets (J=8.64 Hz) at δ 7.507 and 6.971 were assigned to H-5 and H-6, respectively, and exclude the possibility of a C-6 to C-6′ linkage. Assuming oxygenation to be at C-7 in each nucleus, the only structure possible for matsukaze lactone is **6**.

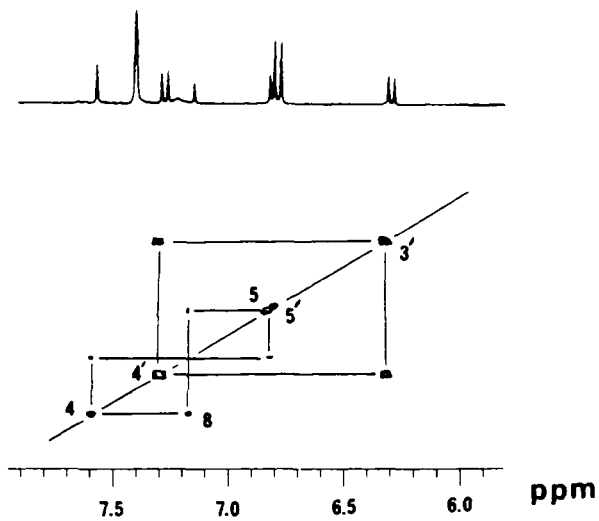
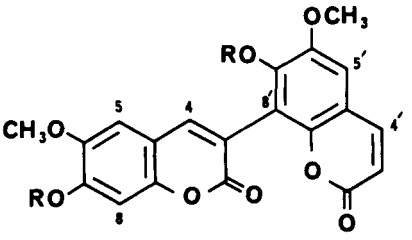
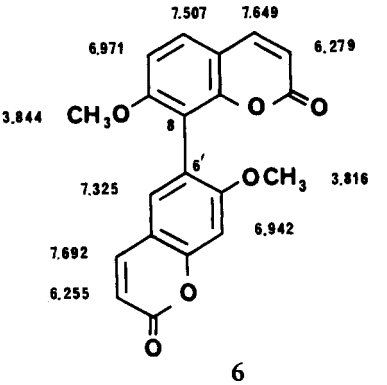


FIGURE 3. COSY spectrum of ipomopsin diacetate in $CDCl_3$-C_6D_6 at 360 MHz (250 msec pulse delay, aromatic region only).



4 R=H

5 R=Ac



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The ambiguities in the proton assignments were resolved through a selective decoupling experiment and a nOe experiment. In the latter, careful irradiation at δ 6.942 (H-8′) caused at 3.9% enhancement in the intensity of the signal at δ 3.816, which can therefore be assigned to the 7′-OCH_3 resonance.[5] Irradiation at δ 7.692 (low power) began to collapse the doublet at δ 6.255, while leaving the companion doublet at δ 6.279 intact. In addition, this irradiation also significantly sharpened the signal at δ 6.942 (H-8′), indicating that the ^5J coupling between H-4′ and H-8′ was being eliminated. Thus, the pairs of doublets can be assigned as δ 7.692 (H-4′) and δ 6.255 (H-3′), and δ 7.649 (H-4) and δ 6.279 (H-3), completing the ^{1}H-nmr assignments of matsukaze-lactone (**6**).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage instrument and are uncorrected. Uv spectra were measured on a Beckman model DB-G grating spectrophotometer, and the ir spectra were obtained on Beckman model 18-A spectrophotometer, with polystyrene calibration at 1601 cm^{-1}. ^{1}H-nmr spectra were recorded on a Varian model T-60A instrument, equipped with a Nicolet model TT-7 Fourier Transform attachment or on a Nicolet NT-360 spectrometer at the University of Illinois at Urbana. TMS was used as an internal standard, and chemical shifts are reported on the δ (ppm) scale. Mass spectra were obtained with a Varian MAT 112S double-focusing spectrometer operating at 70 eV.

PLANT MATERIAL AND INITIAL FRACTIONATION.—The origin of the plant material, its identification, and the initial fractionation of a methanolic extract of the whole plant (10 kg) of *I. aggregata* have been described previously (1).

SEPARATION AND ISOLATION.—The extract (600 g) was treated with $CHCl_3$ and the soluble portion (500 g) chromatographed on silica gel[6] (5 kg), packed in $CHCl_3$, eluting with $CHCl_3$. The $CHCl_3$ eluate (55 g) was further chromatographed on a silica gel column (600 g) eluting successively with $CHCl_3$ and $CHCl_3$ containing increasing volumes of MeOH. A total of 22 fractions (2 liters each) was collected. The eluate from $CHCl_3$ afforded scopoletin (**1**, 15 mg, 0.00015%) from fraction 4, and from fraction 13, eluted with $CHCl_3$-1%-MeOH, ipomopsin (**4**, 30 mg, 0.0003%) was isolated.

IDENTIFICATION OF SCOPOLETIN (**1**).—Scopoletin (**1**) was obtained as colorless needles, mp 199-201°, and was identified by comparison with an authentic sample of scopoletin (1) isolated from *Simaba multiflora* (16). The ^{1}H-nmr spectrum (60 MHz) is summarized in Table 2.

ACETYLATION OF SCOPOLETIN (**1**).—Scopoletin (**1**, 10 mg) was treated with Ac_2O-pyridine (1:1, 0.5 ml) at 100° for 1 h. Work-up in the usual way afforded an acetate (**3**, 9 mg) as colorless needles, mp

[5]Because of the close chemical shift of the resonance of H-6, the 7-OCH_3 is also enhanced (1.9%) under these conditions.

[6]E. Merck, Darmstadt, West Germany.

180-182°; ms *m/z* 234 (M^+, 27%), 192 (100), and 177 (100). The ^{1}H-nmr spectrum (60 MHz) is summarized in Table 2.

TABLE 2. Chemical Shifts of Scopoletin (**1**) and Ipomopsin (**4**) and Their Acetate Derivatives

Proton	Compound					
	1[a]	**3**[b]	**4**[a]	**5**[c]	**Δ=3-1**	**Δ=5-4**
3-H	6.21 (d)	6.40 (d)			+0.19	
4-H	8.06 (d)	7.66 (d)	7.90	7.683	−0.40	−0.22
5-H	7.21	6.97	7.32	7.058	−0.24	−0.26
8-H	6.79	7.08	6.88	7.155 (d)	+0.29	+0.28
3′-H			6.24 (d)	6.410 (d)		+0.17
4′-H			7.97 (d)	7.695 (d)		−0.29
5′-H			7.26	7.008		−0.25
8′-H						
OCH_3	3.84	3.88	3.84	3.880		
			3.90	3.900		
OAc		2.35		2.233		
				2.378		

[a]Obtained at 60 MHz in DMSO-d_6.
[b]Obtained at 60 MHz in $CDCl_3$.
[c]Obtained at 360 MHz in $CDCl_3$.

CHARACTERIZATION OF IPOMOPSIN (**4**).—Pale yellow microneedles, mp over 310°; ir ν max (KBr) 3340, 3010, 2920, 2880, 1700, 1680, 1610, 1580, 1570, 1500, 1485, 1465, 1460, 1450, 1410, 1350, 1295, 1270, 1200, 1150, 1110, 1070, 1040, 965, 950, 920, 870, 850, 835, 815, 795, 780, 765 and 705 cm^{-1}; uv λ max (MeOH) (log ε) 350 (4.39), 303 (sh) (4.24), 257 (sh) (4.23), 203 (sh) (4.36), and 213 nm (4.38), λ max (MeOH + 1N NaOH) 397 nm (4.91); ^{1}H-nmr (60 MHz, DMSO-d_6) δ 3.84 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.24 (1H, d, J=9.2 Hz, 3′-H), 6.88 (1H, s, 8-H), 7.26 (1H, 5′-H), 7.32 (1H, s, 5-H), 7.90 (1H, s, 4-H), and 7.97 ppm (1H, d, J=9.2 Hz, 4′-H); ms, *m/z* 382 (M^+, 100%), 367 (4), 354 (10), 339 (15), 336 (33), 323 (5), 321 (4), 311 (8), 296 (4), 295 (4), 283 (5), 269 (5), 241 (5), 175 (5), 169 (6), 163 (5), 161 (6), 155 (12), 148 (7), 141 (6), 127 (6), 120 (7), 113 (7), 106 (4), 99 (5), 92 (4), 79 (11), 76 (4), 69 (17), 62 (8), and 51 (12). Mass measurement, Obsd. 382.0686; Calcd. for $C_{20}H_{14}O_8$ 382.0687.

ACETYLATION OF IPOMOPSIN (**4**).—Ipomopsin (**4**, 10 mg) was treated with Ac_2O-pyridine (1:1, 0.5 ml) at 100° for 1 h. Work-up in the usual way afforded a diacetate derivative (**5**, 8 mg) as colorless needles; mp 218-221°; ir ν max (KBr) 3000, 2990, 2800, 1765, 1705, 1620, 1585, 1570, 1500, 1470, 1445, 1420, 1410, 1380, 1350, 1310, 1285, 1210, 1200, 1150, 1110, 1085, 1045, 980, 960, 945, 915, 865, 840, 785, 770, 750, and 700 cm^{-1}; ^{1}H-nmr (360 MHz, $CDCl_3$) δ 2.233 (3H, s, 7′-Ac), 2.378 (3H, s, 7-OAc), 3.88 (3H, s, 6-OCH_3), 3.903 (3H, s, 6′-OCH_3), 6.410 (1H, d, J=9.5 Hz, 3′-H), 7.008 (1H, s, 5′-H), 7.058 (1H, 5-H), 7.155 (1H, d, J=0.4 Hz, 8-H), 7.683 (1H, s, 4-H), and 7.695 ppm (1H, d, J=9.5 Hz, 4′-H); ms, *m/z* 466 (M^+, 5%), 424 (25), 383 (22), 382 (100), 381 (4), 339 (4), and 336 (11).

BIOLOGICAL ACTIVITY.—Ipomopsin (**4**) was inactive in the KB and P-388 lymphocytic leukemia test systems *in vitro* (17).

ACKNOWLEDGMENTS

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