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BENZOMALVIN D, A NEW 1,4-BENZODIAZEPINE ATROPISOMER

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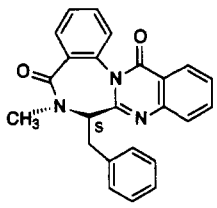
ABSTRACT.—A new compound, benzomalvin D [**5**], isolated from a fungal culture of *Penicillium* sp., is an atropisomer of the previously reported benzomalvin A [**1**]. Benzomalvins A [**1**] and D [**5**] interconvert to form a 4:1 mixture at room temperature and are totally separable by hplc. This equilibrium phenomenon was further explored through total synthesis of benzomalvin A [**1**] and related compounds, leading to the first report of natural atropisomers of 1,4-benzodiazepines.

Recently we reported the isolation of a new series of substance-P inhibitors, benzomalvins A [**1**], B [**2**], and C [**3**], from a fungal culture of *Penicillium* sp. (1). The structures of these compounds were determined using spectroscopic methods, mainly nmr. Benzomalvins resemble structurally the known cholecystokinin antagonist, asperlicin [**4**] (2–5). They possess a quinazolino-benzodiazepine-dione ring skeleton and a 3-substituted benzyl group. Further examination of the culture extract by hplc and ^{1}H-nmr spectroscopy led to the detection and subsequent isolation of a new unstable benzodiazepine, which we have designated as benzomalvin D [**5**].



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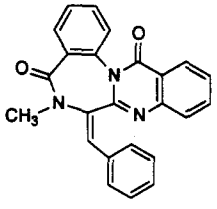

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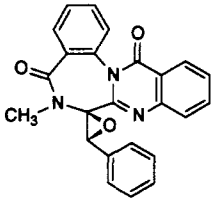

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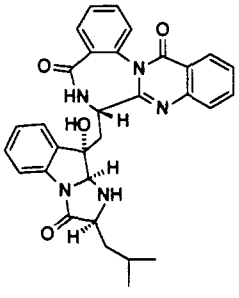

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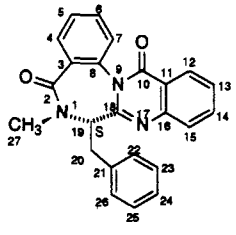

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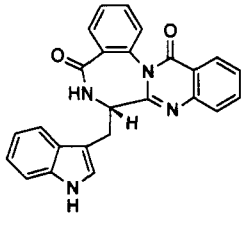

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In solution at room temperature overnight, 80% of **5** converted into benzomalvin A [**1**]. This conversion is reversible whereby **1** slowly reverts back to **5** (20%). After conversion into an equilibrated mixture containing **1** (80%) and

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5 (20%), these compounds were separated using reversed-phase hplc. This interconversion was significantly retarded by storing each compound in solid form at −40°. Since this interconversion was not readily anticipated from a cursory examination of their structures, we undertook a study of this equilibrium phenomenon including the total synthesis of benzomalvin A [**1**] and related compounds. The results are described herein.

Fabms revealed that **5** had the same mol wt as **1**. ^{1}H- and ^{13}C-nmr spectra of **5** showed similar characteristics: one methyl, one methylene, one methine, 13 protonated sp^2, six non-protonated sp^2, and two carbonyl carbons. However, the chemical shifts of the methine (C-19), methylene (C-20), and *N*-methyl (C-27) signals in **5** were significantly different from the same carbons in **1** (Table 1). No clear heteronuclear 2D nmr data for **5** were obtained due to its short life time in various solvents. We thus decided to explore this equilibrium phenomenon first by confirming the structure of **1** through total synthesis.

The total synthesis of **1** (Figure 1) was carried out using a modification of the procedure for the synthesis of asperlicin C [**6**] (6). Reaction of isatoic anhydride [**7**] with L-phenylalanine af-

TABLE 1. Selected ^{1}H- and ^{13}C-Nmr Spectral Data of Compounds **1**, **2**, **5**, and **12**.

Position	1		2		5		Position	12a*		12b*	
	^{13}C δ	^{1}H δ	^{13}C δ	^{1}H δ	^{13}C δ	^{1}H δ		^{13}C δ	^{1}H δ	^{13}C δ	^{1}H δ
2	167.6	—	165.8	—	165.4	—	2	168.4	—	166.1	—
18	153.7	—	151.1	—	153.7	—	10 ...	171.5	—	170.0	—
19	59.3	4.87	128.9	—	70.4	4.75	11 ...	56.3	4.36	68.4	4.28
20	33.7	3.79	132.8	6.72	38.1	2.74	12 ...	32.2	3.49	34.6	2.73
		3.42				2.33			3.22		2.86
27	27.9	3.03	36.1	3.45	36.1	2.91	19 ...	29.3	3.15	39.6	2.92

*12a and 12b represent 2 conformers of 12.

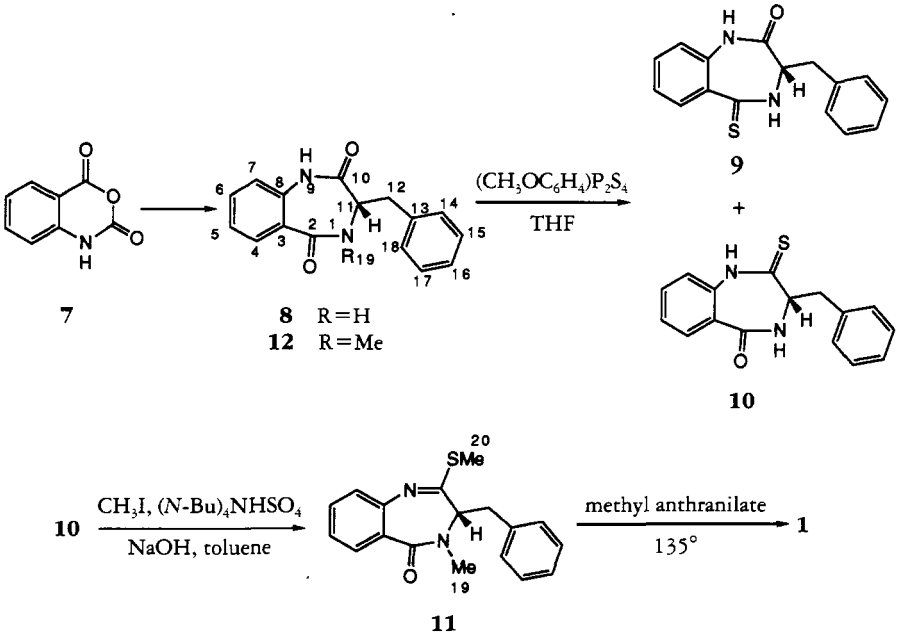

FIGURE 1. Synthesis of benzomalvin A [**1**].

forded the corresponding *N*-anthranoyl-L-phenylalanine derivative. Without isolation this compound was then converted to the benzodiazepinedione **8**. Further elaboration of **8** to give **1** required a regioselective annulation with anthranilic acid. This was accomplished in three steps by first reacting **8** with Lawesson's reagent (7) to give equivalent amounts of the regioisomeric thionamides **9** and **10**, which were separated using Si gel flash chromatography. The desired thionamide **10** was then transformed with iodomethane to the corresponding *N*-methyl, methyl imino thioether **11**. Heating of **11** with methyl anthranilate yielded **1** in 3.7% total yield. The chiral purity of the synthetic compound was established by comparison of the optical rotation with that of the natural product. Other spectroscopic data were identical with those of the natural product. Moreover, the synthetic benzomalvin A exhibited the same phenomenon as the natural product forming **5** in the same percentages in solution.

This interconversion was observed in benzomalvin A [**1**] but not in the related asperlicins. The only structural difference between **1** and asperlicin C [**6**] at the benzodiazepine ring is that **1** contains an *N*-methyl group. To investigate whether the *N*-methyl group causes this equilibrium phenomenon, we synthesized the model compound **12**. The ^{1}H-nmr spectrum of **12** showed clearly the presence of two conformers at a ratio of 60:40, whereas the unmethylated parent compound **8** yielded only one set of ^{1}H-nmr signals. However, the two conformers of **12** are not chromatographically separable, suggesting that the ring inversion in **12** is too rapid to allow for the separation at room temperature, but slow enough for the detection of conformers by nmr. This further suggested that we were also observing conformers for **1**. However, in this case the energy barrier to interconversion is high enough to allow the isolation of two atropisomers [**1** and **5**] at room temperature. A variable temperature ^{1}H-nmr experiment from $-90°$ to $90°$ was conducted on the equilibrated mixture of **1** and **5**. The coalescence of the resonances of **1** and **5** did not occur and the signals of **5** increased from 20% at $27°$ to 27% and 31% at $50°$ and $90°$, respectively. This is strong evidence to substantiate the high energy barrier between **1** and **5**.

Molecular modelling of **1** using the molecular dynamics calculation on QUANTA (8) yielded structure **13** with an equatorial benzyl group and structure **14** with an axial benzyl group. Nmr spectral data of **1** and **5** fit well with structures **13** and **14**, respectively (Figure 2). In **14**, the methylene (C-20) was shielded by an aromatic moiety of the ring resulting in the upfield ^{1}H-nmr shifts at δ 2.33 and 2.74, as compared to δ 3.42 and 3.79 in **1**. The ^{13}C-nmr signals of the methine (C-19) at δ 70.4 and the *N*-methyl (C-27) at δ 36.1 in **5**, appearing at much lower fields than those at δ 59.3 and 27.9 in **1**, implied that the amide bond in **5** possesses more π-bond characteristics than that of **1**. This rationale is supported by the fact that in benzomalvin B [**2**], an analogue with a fully conjugated ring system, the *N*-methyl carbon also absorbed at δ 36.1 (1) (Table 1). Examination of space-filling models of **1** and **5** showed that the bulky quinazolone moiety and the *N*-methyl group restricted drastically the movement of the benzyl group as the 1,4-benzodiazepine ring inverted.

The presence of two atropisomers of benzomalvin A [**1**] is also supported by the finding that benzomalvin B [**2**], a molecule without asymmetric carbons, exhibited optical activity ($[\alpha]_D$ $+158°$) (1). In **2**, the phenyl group is cis to the quinazolone group and the great steric restriction of the two groups may totally prevent the inversion of the benzodiazepine ring, thus allowing the adoption of only one conformer to form a stable atropisomer at room temperature.

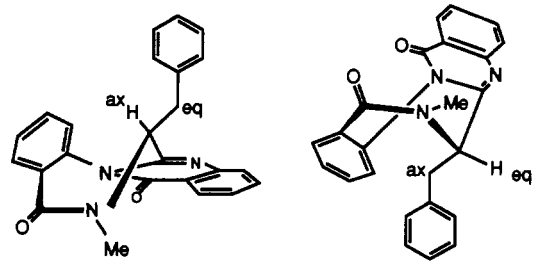

FIGURE 2. Atropisomers of benzomalvins.

Whether the structure of **2** is the conformational enantiomer **15** or **16** must be further determined by X-ray crystallography. A literature precedent for conformational enantiomers of synthetic 1,4-benzodiazepines [**17** and **18**] with bulky *N-tert*-butyl groups also exists (Figure 3) (9). We believe this is the first report of natural atropisomers of 1,4-benzodiazepines including a separation of the two conformational diastereomers, benzomalvins A [**1**] and D [**5**].

EXPERIMENTAL

ISOLATION OF BENZOMALVIN D [**5**].—The whole broth (2 liters) of the microbial culture *Penicillium* sp. was extracted with EtOAc (2 liters×2). After evaporation of solvent *in vacuo*, a residue (520 mg) was obtained. The extract was fractionated by using high-speed centrifugal counter-current chromatography with a solvent system containing hexanes-$CHCl_3$-MeOH-H_2O (5:5:4:3), using the lower layer as stationary phase. The resulting active fractions were further separated using hplc on a reversed-phase C_{18} column with 30% H_2O/MeOH to yield benzomalvin D [**5**] along with benzomalvins A [**1**], B [**2**], and C [**3**]. Compound **5** was isolated as a white solid; $[\alpha]^{25}D$ +48°; ^{1}H-nmr ($CDCl_3$) δ 2.33 (1H, dd, J=13.8 and 11.3 Hz), 2.74 (1H, dd, J=13.8 and 5.9 Hz), 2.91 (3H, s), 4.75 (1H, dd, J=11.3 and 5.9 Hz), 6.96 (2H, dd, J=7.6 and 1.5 Hz), 7.20~7.28 (3H, m), 7.53 (1H, td, J=7.6 and 1.5 Hz), 7.59–7.67 (4H, m), 7.79 (1H, td, J=7.6 and 1.5 Hz), 8.01 (1H, dd, J=7.6 and 1.5 Hz), 8.32

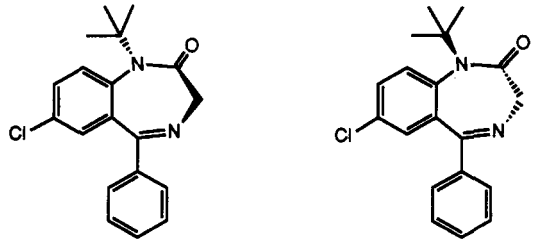

FIGURE 3. Enantiomers **17** and **18**.

(1H, dd, J=7.6 and 1.5 Hz); ^{13}C nmr ($CDCl_3$) δ 35.9 (t), 38.3 (q), 70.3 (d), 121.4 (s), 127.3 (d), 127.4 (d), 127.5 (d), 127.7 (d), 128.1 (d), 128.8 (d, 3C), 129.0 (d), 130.7 (d), 131.1 (d), 132.1 (s), 133.0 (s), 135.0 (d), 135.9 (s), 146.3 (s), 153.7 (s), 161.5 (s), 165.4 (s).

SYNTHESIS OF BENZODIAZEPINEDIONE **8**.—Isatoic anhydride (25 g), phenylalanine (25 g), triethylamine (20 ml), and (120 ml), were stirred at room temperature overnight. The solvent was removed *in vacuo* and glacial HOAc (240 ml) was added to the resulting oil. This solution was then stirred at reflux overnight. The HOAc was then removed *in vacuo* and the residue partitioned between EtOAc and H_2O. The organic layer was washed with $NaHCO_3$ followed by H_2O. The organic layer was then dried with Na_2SO_4 and the solvent removed *in vacuo* to afford a white powder (39.5 g, 96%): mp 238–242°; $[\alpha]^{25}$D +185.3° (c=0.57, MeOH); uv (MeOH) λ max (ε) 218 (26,800), 293 (2,760) nm; ir ($CHCl_3$) ν max 3180, 3085, 3065, 3030, 1680, 1660, 1605, 1490, 1450, 1410, 755, 700 cm^{-1}; ^{1}H nmr [$(CD_3)_2SO$] δ 10.40 (1H, s, H-9), 8.50 (1H, d, J=6.1 Hz, H-1), 7.69 (1H, dd, J=7.9 and 1.1 Hz, H-4), 7.49 (1H, dt, J=1.5 and 7.0 Hz, H-6), 7.31 (2H, d, J=7.2 Hz, H-14, H-18), 7.24 (2H, t, J=7.1 Hz, H-15, H-17), 7.18 (1H, t, J=7.3 Hz, H-5), 7.17 (1H, m, H-16), 7.11 (1H, d, J=8.0 Hz, H-7), 3.91 (1H, dt, J=9.2 and 5.6 Hz, H-11), 3.15 (1H, dd, J=14.2 and 5.3 Hz, H-12a), 2.88 (1H, dd, J=14.2 and 4.2 Hz, H-12b); ^{13}C nmr [$(CH_3)_2SO$] δ 167.7 (C-2), 126.3 (C-3), 130.3 (C-4), 123.9 (C-5), 132.2 (C-6), 120.9 (C-7), 136.7 (C-8), 171.2 (C-10), 53.9 (C-11), 33.3 (C-12), 137.9 (C-13), 129.3 (2C, C-14), 128.1 (2C, C-15), 128.3 (C-16); hrfabms m/z [MH]$^+$ 267.1147 (calcd for $C_{12}H_{13}N_4O_4$ 267.1134).

CONVERSION OF **8** TO THIOAMIDES **9** AND **10**.—The benzodiazeinedione **8** (5 g) and Lawesson's reagent (7.5 g) were stirred in tetrahydrofuran (110 ml) for 3 h. The solvent was removed *in vacuo* and the regioisomeric products separated using Si gel flash chromatography. The less polar isomer **9** was obtained as a yellow solid (1.6 g, 30%); $[\alpha]^{25}$D +167° (c=1.0, [$CH_3)_2CO$]; mp 110–114°; uv λ max (ε) 219 (11,630), 320 (2,990) nm; ir ($CHCl_3$) ν max 3225, 2840, 1685, 1605, 1520, 1500, 1475, 1025, 755, 410, 405 cm^{-1}; ^{1}H nmr [$(CD_3)_2SO$] δ 10.78 (1H, d, J=6.3 Hz, H-1), 10.54 (1H, s, H-9), 7.94 (1H, d, J=7.8 Hz, H-4), 7.48 (1H, dt, J=1.3 and 7.5 Hz, H-6), 7.32 (2H, br d, J=7.0 Hz, H-14, H-18), 7.25 (2H, t, J=7.3 Hz, H-15, H-17), 7.18 (1H, m, H-16), 7.17 (1H, m, H-5), 7.06 (1H, d, J=8.0 Hz, H-7), 4.06 (1H, dt, J=9.0 and 6.0 Hz, H-11), 3.28 (1H, m, H-12b), 3.10 (1H, m, H-12a); ^{13}C nmr [$(CD_3)_2SO$] δ 176.6 (C-2), 127.6 (C-3), 132.1 (C-4), 123.8 (C-5), 132.4 (C-6), 121.0 (C-7), 132.3 (C-8), 169.6 (C-10), 58.5 (C-11), 32.3 (C-12), 137.5 (C-13), 129.2 (2C, C-14, C-18), 128.2 (2C, C-15, C-17), 126.4 (C-16); hrfabms m/z [MH]$^+$ 283.0912 (calcd for $C_{12}H_{13}N_4OS$, 283.0905).

The more polar isomer **10** was obtained as a yellow solid (1.5 g, 28%): mp 105–110°; $[\alpha]^{25}$D +200° [c=1.0, $(CH_3)_2CO$]; uv (MeOH) λ max (ε) 210 (10,950), 311 (6,680) nm; ir ($CHCl_3$) ν max 3170, 3065, 3030, 1655, 1605, 1588, 1575, 1480, 1395, 1025, 780, 410 cm^{-1}; ^{1}H nmr [$(CD_3)_2SO$] δ 12.38 (1H, s, H-9), 8.68 (1H, d, J=6.8 Hz, H-1), 7.69 (1H, br d, J=7.0 Hz, H-4), 7.57 (1H, dt, J=1.5 and 7.1 Hz, H-6), 7.28 (2H, m, H-14, H-18), 7.28 (2H, m, H-15, H-17), 7.28 (1H, m, H-16), 7.22 (1H, m, H-5), 7.21 (1H, m, H-7), 4.11 (1H, br s, H-11), 3.28 (1H, br s, H-12a), 3.03 (1H, br s, H-12b); ^{13}C nmr [$(CD_3)_2SO$] δ 168.3 (C-2), 127.5 (C-3), 130.2 (C-4), 126.3 (C-5), 132.2 (C-6), 121.3 (C-7), 136.4 (C-8), 169.3 (C-10), 57.3 (C-11), 37.0 (C-12), 140.7 (C-13), 129.3 (2C, C-14, C-18), 128.1 (2C, C-15, C-17), 125.7 (C-16); hrfabms m/z [MH]$^+$ 283.0919 (calcd for $C_{12}H_{13}N_4OS$, 283.0905).

METHYLATION OF **10** TO FORM **11**.—The thioanamide **10** (3.5 g) was dissolved in toluene (200 ml). NaOH (40%, 200 ml), MeI (6 ml), and $(n\text{-Bu})_4NHSO_4$ were added sequentially. After this mixture was stirred for 20 min at room temperature, EtOAc was added and the aqueous layer removed. The organic layer was washed with 10% citric acid/H_2O (2×) and then H_2O (1×), the organic layer dried over Na_2SO_4, and the solvent removed *in vacuo*. The residue was purified using flash Si gel chromatography to give **11** as a clear yellow oil (1.1 g, 29%): [α]D −352.6° (c=0.95, CH_2Cl_2); uv (MeOH) λ max (ε) 210 (19,130), 232 (13,640), 310 (4,220) nm; ir ($CHCl_3$) ν max 3065, 3030, 2930, 1690, 1620, 1595, 1500, 1470, 1455, 1395, 1340, 1120, 1045, 760, 700, 410 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 8.08 (1H, dd, J=8.0 and 1.4 Hz, H-4), 7.54 (1H, dt, J=7.5 and 1.4 Hz, H-6), 7.32 (1H, m, H-7), 7.29 (1H, m, H-5), 7.29 (1H, m, H-16), 7.28 (2H, t, J=6.8 Hz, H-15, H-17), 6.99 (2H, d, J=6.6 Hz, H-14, H-18), 3.91 (1H, t, J=8.3 Hz, H-11), 2.91 (3H, s, H-19), 2.66 (2H, d, J=8.3 Hz, H-12), 2.49 (3H, s, H-20); ^{13}C nmr ($CDCl_3$) δ 167.1 (C-2), 127.5 (C-3), 131.9 (C-4), 131.0 (C-5), 126.2 (C-6), 125.1 (C-7), 145.2 (C-8), 166.6 (C-10), 67.8 (C-11), 34.3 (C-12), 136.3 (C-13), 129.0 (2C, C-14, C-18), 128.7 (2C, C-15, C-17), 127.1 (C-16), 39.2 (C-19), 14.2 (C-20); hrfabms m/z [MH]$^+$ 311.1206 (calcd for $C_{17}H_{18}N_4OS$, 311.1218).

SYNTHESIS OF BENZODIAZEPINEDIONE **12**.—Isatoic anhydride (910 mg), *N*-methyl phenylalanine (1 g), triethylamine (1 ml), and H_2O (10 ml), were reacted by the general procedure described for **8**. Workup as described in the synthesis of **8**

afforded an oil (680 mg, 39%). This material (97 mg) was purified by a C_{18} hplc column with 30% H_2O/MeOH to give **12** (33 mg) as a colorless oil: $[\alpha]_D$ −70° (c=1.0, MeOH); ^{1}H nmr ($CDCl_3$) δ 2.72 (0.4H, dd, J=14.5 and 8.5 Hz), 2.86 (0.4H, dd, J=14.5 and 7.5 Hz), 3.22 (0.6H, dd, J=14.5 and 7.5 Hz), 3.50 (0.6H, dd, J=14.5 and 7.5 Hz), 4.28 (0.4H, dd, J=8.5 and 7.5 Hz), 4.36 (0.6H, dd, J=7.5 and 7.5 Hz), 6.94–7.09 (m), 7.19–7.38 (m), 7.45 (t), 7.53 (t), 7.98 (0.6H, d, J=7.5 Hz), 8.12 (0.4H, d, J=7.5 Hz), 9.04 (0.6H, br s), 9.38 (0.4H, br s); ^{13}C nmr ($CDCl_3$) δ 29.3, 32.2, 34.6, 39.6, 56.3, 68.4, 120.1, 120.7, 125.2, 125.3, 126.7, 126.9, 127.0, 127.1, 127.3, 128.0, 128.7, 128.8, 128.9, 129.0, 130.0, 131.4, 131.8, 132.4, 132.7, 134.6, 135.5, 135.6, 136.4, 166.1, 168.4, 170.0, 171.5.

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