Isorawsonol and Related IMP Dehydrogenase Inhibitors from the Tropical Green Alga Avrainvillea rawsonii

Jian Lu Chen, William H. Gerwick, Randy Schatzman, and Maureen Laney

J. Nat. Prod., **1994**, 57 (7), 947-952• DOI:
10.1021/np50109a011 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 7, pp. 947-952, July 1994

ISORAWSONOL AND RELATED IMP DEHYDROGENASE INHIBITORS FROM THE TROPICAL GREEN ALGA *AVRAINVILLEA RAWSONII*

JIAN LU CHEN,[1] WILLIAM H. GERWICK,*

College of Pharmacy, Oregon State University, Corvallis, Oregon 97331

RANDY SCHATZMAN, and MAUREEN LANEY

Institute of Biochemistry and Cell Biology, Syntex Research, Palo Alto, California 94304

ABSTRACT.—Guided by inhibitory activity against IMP dehydrogenase (IMPDH), a new brominated diphenylmethane derivative, isorawsonol, has been isolated from the tropical green alga *Avrainvillea rawsonii*. Its structure was determined by detailed spectroscopic analysis including HMQC and HMBC. The known metabolites, avrainvilleol, avrainvilleol methyl ether, 3-bromo-4,5-dihydroxylbenzyl alcohol, and rawsonol, were also isolated from this alga and two of these showed a modest ability to inhibit IMPDH. The formation of isorawsonol is envisioned as being similar to that of rawsonol involving condensation of two molecules of avrainvilleol.

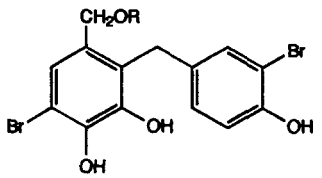
Tropical marine Chlorophytes (green algae) are known for their production of linear oxygenated (1) and brominated (2) terpenes. However, members of the genus *Avrainvillea* (Halimedaceae) have been shown characteristically to produce brominated diphenylmethane derivatives. Avrainvilleol [**1**] and its methyl ether [**2**] were isolated from *A. longicaulis* (3) while investigations of *A. nigricans* (4) resulted in the isolation of avrainvilleol, 5′-hydroxyisoavrainvilleol [**3**], and 3-bromo-4,5-dihydroxybenzyl alcohol [**4**]. Rawsonol [**5**] was reported from *A. rawsonii* as an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (5). Herein, we describe our findings of potential anticancer-type activity from *A. rawsonii* using a mechanism-based enzyme assay based on inosine 5′-monophosphate dehydrogenase (IMPDH), a rate-limiting enzyme in purine biosynthesis which is responsible for the formation of xanthosine 5′-monophosphate (XMP) from IMP (6). The activity of IMPDH has been linked with cellular proliferation (7) and inhibition of the enzyme has been demonstrated to have anticancer and immunosuppressive effects (8,9). Our attempts to identify novel inhibitors of IMPDH have resulted in the isolation of a new tetraphenyl natural product, isorawsonol [**6**], as well as the previously reported metabolites **1**, **2**, **4**, and **5**. Three of these diphenylmethane derivatives were modestly inhibitory to IMPDH.

RESULTS AND DISCUSSION

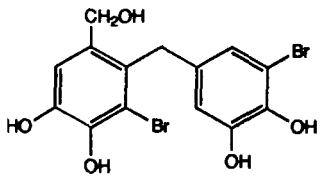
The $CHCl_3$-MeOH (2:1) extract of *A. rawsonii* showed inhibitory activity against IMPDH (IC_{50}=4.22 μg/ml). Gradient flash Si gel cc of the extract led to the fractionation of the IMPDH-active components (6). The non-polar active fractions (hexanes to 10% EtOAc/hexanes) were shown to contain elemental sulfur (S_8), which was responsible for the bioactivity, and C-16–C-18 hydrocarbons dominated by 17:0 and 17:1 species. The polar active fractions (70% EtOAc/hexanes to 30% MeOH/EtOAc) were subjected to further chromatography on Si gel. Four previously described diphenylmethane derivatives [**1**, **2**, **4**, **5**], and one new analogue [**6**], were isolated, three as modestly active IMPDH inhibitors (**1**, IC_{50}=18 μM; **2**, inactive; **4**, inactive; **5**, IC_{50}=ca. 10 μM; **6**, IC_{50}=7.4 μM).

The molecular formula of the new compound **6** was $C_{28}H_{22}Br_4O_7$, as determined by negative-ion hrfabms. Detailed analysis of the ^1H- and ^{13}C-nmr spectra, aided by HMQC (10), and HMBC (11) experiments, enabled establishment of the structure of **6**.

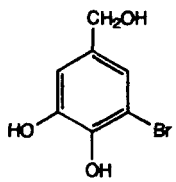
[1]Current address: Shaman Pharmaceuticals, Inc., 213 East Grand Ave., South San Francisco, CA 94080.



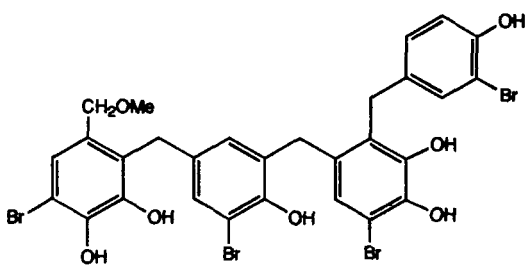
1 R=H
2 R=Me



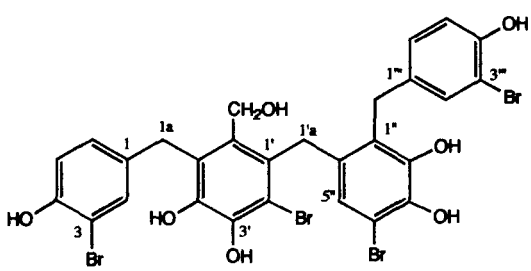
3



4



5



6

Examination of the ^{1}H-nmr spectrum (Figure 1) of **6** indicated the presence of two 1,3,4-trisubstituted aromatic rings: (1) δ 7.40, 1H, d, J=1.9 Hz; δ 7.14, 1H, dd, J=8.3 and 1.9 Hz; δ 6.91, 1H, d, J=8.3 Hz; (2) δ 7.31, 1H, d, J=1.6 Hz; δ 7.00, 1H, dd, J=8.2 and 1.6 Hz; δ 6.87, 1H, d, J=8.2 Hz), and one pentasubstituted aromatic ring (δ 6.14, 1H, s). A fourth fully substituted aromatic ring was deduced from consideration of ^{13}C-nmr data and the molecular formula.

The two trisubstituted rings were shown to each possess a hydroxyl, bromine, and methylene substituent (δ 4.14, 2H, s and 4.17, 2H, s) by comparison of ^{13}C-nmr shifts with model compounds (3–5) and from a combination of HMQC and HMBC data (Figure 2). The substitution pattern of these groups, deduced by these same data, were consistent with those found in co-metabolites **1**, **2**, and **5**. By deduction, these two rings had to form the two termini of this essentially tetrameric structure. The substituents of the pentasubstituted ring were similarly shown to be a bromine, two hydroxyls, and two methylene groups. HMBC data were used to position the singlet proton of this ring between bromine and methylene substituents (Figure 2). Furthermore, long-range coupling between a quaternary carbon at δ 126.03 and this proton as well as to the bis-benzylic methylenes at δ 4.14 and 4.17 served to orient this ring relative to the other structural subunits.

The final aromatic ring was fully substituted with a bromine atom, two hydroxyls, and three methylene groups. The positions of the three methylene groups were

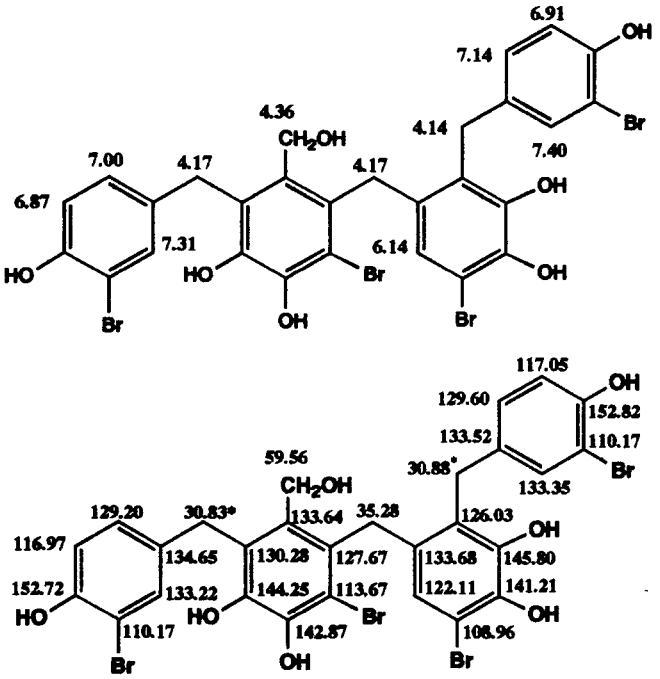


FIGURE 1. Assignment of ^{1}H- and ^{13}C-nmr data for isorawsonol [**6**] from HMQC and HMBC data and from comparisons with data for model compounds [**1–5**] (asterisks indicate assignments may be switched).

determined by HMBC in which an oxygenated methylene (δ 4.36, 2H, s) only showed correlations to aromatic carbons substituted by carbon (δ 127.67, 130.28, and 133.64). Conversely, the flanking bis-benzylic methylene protons (δ 4.17) showed correlations to these same three carbon substituted positions as well as to bromine (δ 113.67) and oxygen (δ 144.25) substituted positions (Figures 1 and 2). Orientation of substituents in this fourth ring was deduced by comparison of ^{13}C-nmr shifts (all in Me_2CO-d_6) of the bis-benzylic methylene carbons with that of avrainvilleol [**1**] and isoavrainvilleol [**3**]. In metabolite **1**, this carbon is relatively shielded by the ortho-hydroxyl functionality and resonates at δ 30.23 (see Experimental),

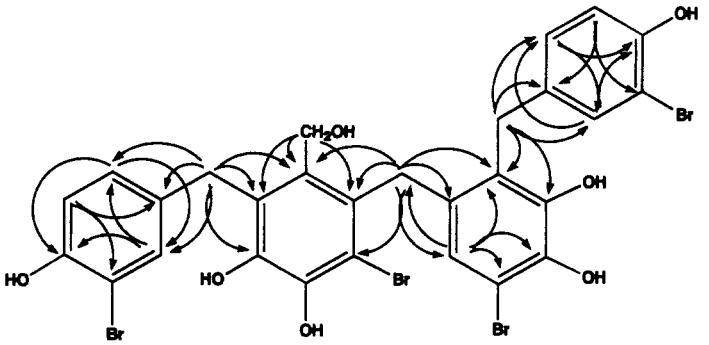


FIGURE 2. Long-range correlations observed by HMBC in isorawsonol [**6**].

whereas, in metabolite **3**, this carbon resonates further downfield at δ 36.25 (4). Apparently, the ortho-bromine in **3** does not shield this position as highly. In isorawsonol [**6**], bis-benzylic carbon-1a resonates at δ 30.83 or 30.88 (Figure 1) while bis-benzylic carbon-1′a resonates at δ 35.28. Hence, this half of the molecule of isorawsonol [**6**] derives from a subunit with the 'avrainvilleol' rather than 'isoavrainvilleol' bromine and hydroxyl substitution pattern.

Energy minimization of isorawsonol (Chem 3D Plus) positioned the singlet aromatic proton (H-5″) in the shielding region of the adjacent aromatic ring, thus helping to explain its anomalously high field chemical shift (δ 6.14, Figure 3). The structure of isorawsonol may be biogenetically rationalized as deriving from two avrainvilleol [**1**] subunits through reactions analogous to those proposed for rawsonol (5) but involving the two more highly substituted rings (Figure 4).

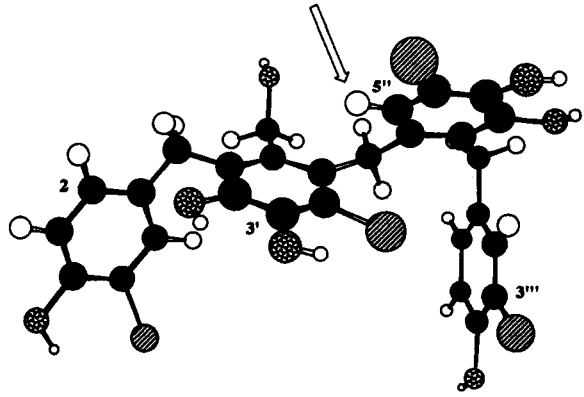


FIGURE 3. Energy-minimized conformation of isorawsonol [**6**] using Chem 3D Plus (white=hydrogen, black=carbon, stippled=oxygen, striped=bromine. Arrow indicates H-5″, which is at anomolously high field (δ 6.14) in the ^{1}H-nmr spectrum, presumably due to shielding effects of the adjacent aromatic ring).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on Bruker AM 400 and AC 300 spectrometers. ^{1}H-Nmr chemical shifts are referenced to Me_2CO-d_6 to the CHD_2OCD_3 residual signal (2.04 ppm). ^{13}C-Nmr chemical shifts are referenced to Me_2CO-d_6 to the solvent signal (29.8 ppm). Mass spectra were recorded on Kratos MS 50 TC and Finnigan 4023 mass spectrometers. Uv spectra were recorded on a Hewlett-Packard 8452A uv-vis spectrophotometer and ir spectra on a Nicolet 510 spectrophotometer. Merck aluminum-backed tlc sheets were used for tlc, and all solvents were distilled from glass prior to use.

PLANT MATERIAL AND EXTRACTION.—The alga *A. rawsonii* (Dickie) Howe was collected on 21 December, 1991, from Baby Beach, Aruba, Netherlands Antilles, and stored in *i*-PrOH at low temperature until workup. A voucher specimen is available from W.H.G. (collection NBB-21 Dec. 91–5). A total of 230 g (dry wt) of the alga was extracted with $CHCl_3$-MeOH (2:1) three times to give the crude extract (5.4 g) which inhibited IMPDH with an IC_{50}=4.22 μg/ml.

BIOASSAY FOR INHIBITORY ACTIVITY TO INOSINE 5′-MONOPHOSPHATE DEHYDROGENASE (IMPDH).—Inhibition of IMPDH by the crude extract, chromatography fractions, and pure compounds was determined as detailed by Ikegami *et al.* (6). Briefly, the assay utilized purified recombinant human IMPDH as an enzyme source and activity was determined spectrophotometically by monitoring formation of NADH at 340 nm.

FRACTIONATION.—The crude extract (5.2 g) was fractionated using flash Si gel cc with a stepwise gradient of hexanes/EtOAc and EtOAc/MeOH to give ten fractions [number, solvent, weight, IMPDH IC_{50}

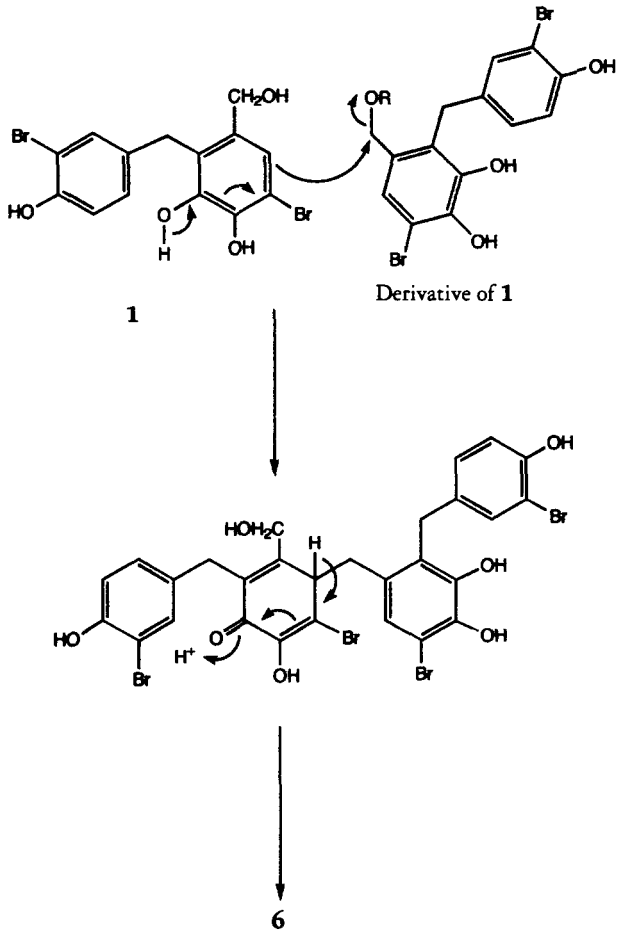


FIGURE 4. Proposed biogenesis of isorawsonol [**6**] from avrainvilleol [**1**].

(μg/ml)]: fraction 1, hexanes, 0.028 g, 0.65 μg/ml; fraction 2, 10% EtOAc/hexanes, 0.67 g, 1.03 μg/ml; fraction 3, 30% EtOAc/hexanes, 1.4 g, 6.5 μg/ml; fraction 4, 50% EtOAc/hexanes, 1.6 g, 6.7 μg/ml; fraction 5, 70% EtOAc/hexanes, 0.27 g, 1.9 μg/ml; fraction 6, 90% EtOAc/hexanes, 0.38 g, 2.8 μg/ml; fraction 7, EtOAc, 0.17 g, 4.2 μg/ml; fraction 8, 30% MeOH/EtOAc, 1.0 g, 2.6 μg/ml; fraction 9, 50% MeOH/EtOAc, 0.27 g, 11.5 μg/ml; fraction 10, MeOH, 0.15 g, 23.6 μg/ml. Hplc (Si, hexanes) and gcms analysis indicated fractions 1 and 2 contained elemental sulfur (S_8) and C-16–C-18 hydrocarbons dominated with 17:0 and 17:1 species.

ISOLATION OF BIOACTIVE DIPHENYLMETHANE DERIVATIVES.—Repetitive chromatography of fractions 5–8 over Si gel with 4% MeOH/$CHCl_3$ gave sequentially the known compounds (identified in each case by comparison of published data with ^{1}H-nmr data, and for **2**, ^{13}C-nmr data as well), avrainvilleol methyl ether [**2**], avrainvilleol [**1**], 3-bromo-4,5-dihydroxybenzyl alcohol [**4**], and rawsonol [**5**], and a new compound, isorawsonol [**6**], as the most polar natural product, all isolated in 5–20 mg quantities. To provide ^{13}C-nmr data for **1** that would be comparable with isoavrainvilleol [**3**] (4) and isorawsonol [**6**], a ^{13}C-nmr spectrum and DEPT-135 were recorded for **1** (300 MHz, Me_2CO-d_6) δ 152.78 (s), 145.68 (s), 142.10 (s), 135.10 (s), 134.14 (s), 133.24 (d), 129.42 (d), 125.66 (s), 122.68 (d), 116.95 (d), 110.10 (s), 108.52 (s), 62.02 (t), 30.23 (t).

Isorawsonol [**6**].—Isorawsonol [**6**] exhibited the following spectral characteristics: uv (MeOH) λ max 224 (ε 23000), 286 nm (ε 8900); ir (neat) ν max 3450, 2930, 2880, 2800, 1640, 1500, 1290 cm^{-1}; negative-ion fabms, 3-nitrobenzyl alcohol matrix, *m/z* $[M-H]^-$ cluster 785 (6)/787 (16)/789 (25)/791 (15)/793 (2), $[M-H-H_2O]^-$ cluster 767/769/771/773/775, $[M-H-Br]^-$ cluster 706/708/710/712, 455 (1)/457 (5)/459 (6)/461 (2), 305 (46)/307 (45), 234 (8), 199 (30), 168 (25), 153 (100), 138 (12), 121 (20), 79 (11)/81

(10), 46 (75); negative-ion hrfab mass measurement for $C_{28}H_{21}{}^{79}Br_2{}^{81}Br_2O_7$, 788.79800 ($\Delta=-0.3$ mass units); positive-ion fabms, *m/z* $[M]^+$ ion cluster 786 (4)/788 (6)/790 (7)/792 (5)/794 (4), $[M-H_2O]^+$ ion cluster 768 (6)/770 (12)/772 (15)/774 (13)/776 (6), 613 (15), 583 (10)/585 (28)/587 (28)/589 (11), 460 (100), 339 (48)/341 (82)/343 (48); ^{1}H nmr (300 MHz, Me_2CO-d_6) δ 7.40 (1H, d, J=1.9 Hz), 7.31 (1H, d, J=1.6 Hz), 7.14 (1H, dd, J=8.3 and 1.9 Hz), 7.00 (1H, dd, J=8.2 and 1.6 Hz), 6.91 (1H, d, J=8.3 Hz), 6.87 (1H, d, J=8.2 Hz), 6.14 (1H, s), 4.36 (2H, s), 4.17 (4H, s), 4.14 (2H, s), see Figure 1 for assignments; ^{13}C nmr (75 MHz, Me_2CO-d_6) δ 152.82 (s), 152.72 (s), 145.80 (s), 144.25 (s), 142.87 (s), 141.21 (s), 134.65 (s), 133.68 (s), 133.64 (s), 133.52 (s), 133.35 (d), 133.22 (d), 130.28 (s), 129.60 (d), 129.20 (d), 127.67 (s), 126.03 (s), 122.11 (d), 117.05 (d), 116.97 (d), 113.67 (s), 110.17 (2C, s), 108.96 (s), 59.56 (t), 35.28 (t), 30.88 (t), 30.83 (t), see Figure 1 for assignments.

ACKNOWLEDGMENTS

We thank R. Kohnert, Oregon State University, Department of Chemistry, for help with nmr (Bruker AM 400 [National Science Foundation (CHE-8216190)] and M.J. Murdock Charitable Trust), and Bruker ACP 300 spectrometers (NIH RR 04039 and NSF CHE-8712343) and B. Arbogast and D. Griffin, Oregon State University, College of Agricultural Chemistry, for help with low-and high-resolution mass spectra [Kratos MS 50 TC (NIH DRR 1S10RR01409)]. We also thank Dale Nagle for running some of the nmr spectra. This research was supported by NIH CA52955.

LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **1**, 251 (1984).
2. D.J. Faulkner, *Nat. Prod. Rep.*, **8**, 97 (1991).
3. H.H. Sun, V.J. Paul, and W. Fenical, *Phytochemistry*, **22**, 743 (1983).
4. M. Colon, P. Guevara, and W.H. Gerwick, *J. Nat. Prod.*, **50**, 368 (1987).
5. B.K. Carté, N. Troupe, J.A. Chan, J.W. Westley, and D.J. Faulkner, *Phytochemistry*, **28**, 2917 (1989).
6. T. Ikegami, Y. Natsumeda, and G. Weber, *Anal. Biochem.*, **150**, 155 (1985).
7. Y. Natsumoda, T. Ikegami, K. Murayama, and G. Weber, *Cancer Res.*, **48**, 507 (1988).
8. M.S. Lui, M.A. Faderan, J.J. Liepniks, Y. Natsumeda, E. Olah, H.N. Jayaram, and G. Weber, *J. Biol. Chem.*, **259**, 5078 (1984).
9. R.E. Morris, J. Wang, J.R. Blum, T. Flavin, M.P. Murphy, S.J. Almquist, N. Chu, Y.L. Tam, M. Kaloostian, A.C. Allison, and E.M. Eugui, *Transplant. Proc.*, **23**, 19 (1991).
10. A. Bax and S. Subramanian, *J. Magn. Res.*, **67**, 565 (1986).
11. A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).

Received 7 February 1994