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RAWSONOL, AN INHIBITOR OF HMG-CoA REDUCTASE FROM THE TROPICAL GREEN ALGA *AVRAINVILLEA RAWSONI*

BRAD K. CARTE,* NELSON TROUPE, JAMES A. CHAN, JOHN W. WESTLEY and D. JOHN FAULKNER†

Department of Biomolecular Discovery, Smith Kline & French Laboratories, Research and Development, P.O. Box 1539, King of Prussia, PA 19406-0939, U.S.A.; †Scripps Institution of Oceanography (A-012F) La Jolla, CA 92093-0212, U.S.A.

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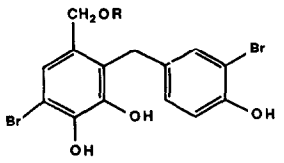
Key Word Index—*Avrainvillea rawsoni*; Udoteaceae; brominated diphenyl methanes; HMG-CoA reductase inhibitor.

Abstract—A novel brominated diphenyl methane derivative, rawsonol, has been isolated from the green alga *Avrainvillea rawsoni*, and its structure determined by chemical and spectral methods. Rawsonol which is a methyl ether formed via condensation/methylation of two molecules of the known metabolite avrainvilleol, inhibited the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.

INTRODUCTION

In the course of our search for inhibitors of cholesterol biosynthesis from natural sources [1], the crude extract of the green alga *Avrainvillea rawsoni* (Udoteaceae, Chlorophyta) was shown to inhibit the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is the enzyme responsible for the formation of mevalonate in the rate-determining step of cholesterol biosynthesis.

While members of the green algae (chlorophyta) are well known for the production of linear oxygenated terpenes [2], the genus *Avrainvillea* has been shown to be almost unique among the green algae because of its ability to produce brominated diphenylmethane derivatives. The major metabolite of an ether extract of *A. longicaulis* [3] was avrainvilleol, (**1**), but extraction with methanol gave the methyl ether **2**. Studies of *A. nigricans* [4] resulted in the isolation of avrainvilleol (**1**), 5′-hydroxyisoavrainvilleol (**3**), and 3-bromo-4,5-dihydroxybenzyl alcohol (**4**). Our present studies have resulted in the isolation of rawsonol (**5**), as the HMG-CoA reductase inhibitory principle of *A. rawsoni*.



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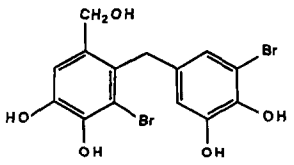
2 R=Me



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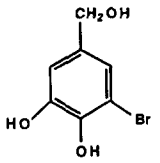

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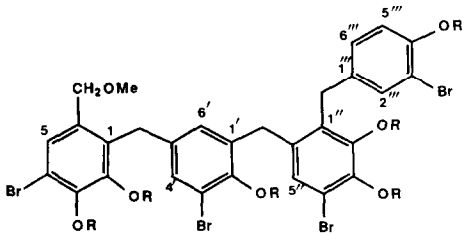


5 R=H
6 R=Ac

RESULTS AND DISCUSSION

By using a bioassay directed isolation procedure guided by activity against HMG-CoA reductase, rawsonol (**5**) was isolated as the active principle (0.01% dry wt) of a methylene chloride–methanol (1:1) extract of *A. rawsoni*. The phenolic nature of **5** was suggested by the broad hydroxyl stretch in the IR spectrum (γ_{OH} = 3000–3600 cm^{-1}) and confirmed by the characteristic phenolic UV absorption (λ_{max}^{MeOH} = 285 nm, ε = 3200) which underwent a bathochromic shift in basic media ($\lambda_{max}^{MeOH+NaOH}$ = 300 nm, ε = 3900). The low resolution FAB

*Author to whom correspondence should be addressed.

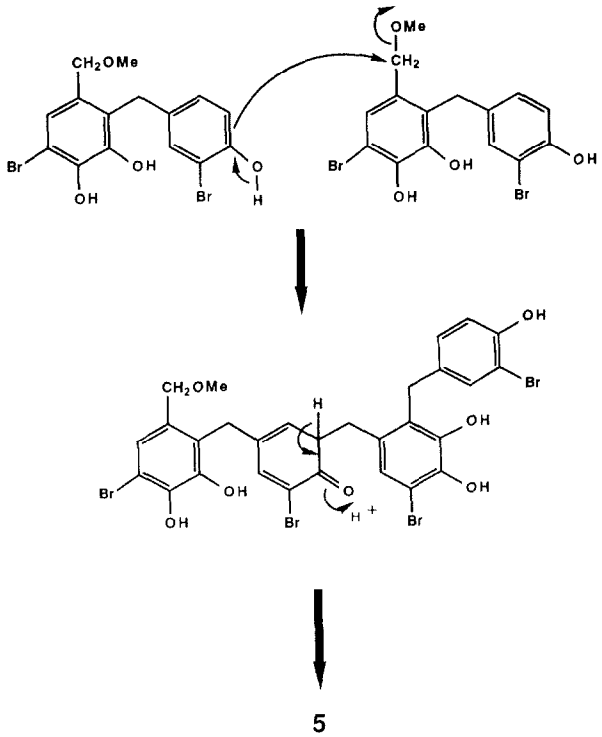
mass spectrum exhibited a parent ion cluster at *m/z* 800/802/804/806/808 indicative of a tetrabromo compound. From these data it appeared that rawsonol (**5**) might have resulted from the dimerization of avrainvilleol methyl ether (**2**) with elimination of a molecule of methanol.

Rawsonol (**5**) proved to be highly unstable and rapidly polymerized to an insoluble black tar. Due to the limited sample size, the compound was stabilized by acetylation (acetic ahydride–pyridine) to give a hexa-acetyl derivative **6** which was analysed by mass spectrometry and NMR. High resolution FAB mass spectrum of rawsonol hexa-acetate (**6**) established a molecular formula of $C_{41}H_{36}Br_4O_{13}$. Rawsonol, $C_{29}H_{24}Br_4O_7$, must therefore have six phenolic hydroxyl groups. The ^{13}C NMR spectrum of **6** showed six acetate carbonyl and methyl signals, one methoxyl group, 24 aromatic signals (7 methines, 17 quaternary carbons) and four methylene groups, of which three were bisbenzylic methylenes (δ31.8 *t*, 32.0 *t*, 35.8 *t*) and one was a benzylic methylene bearing an oxygen substituent (δ68.8 *t*).

Examination of the aromatic region of the ^1H NMR spectrum of **6** indicated the presence of two pentasubstituted aromatic rings (δ6.64, 1H, *s*; 7.00, 1H, *s*), one tetrasubstituted aromatic ring (7.26, 1H, *d*, $J = 1.96$ Hz; 7.40, 1H, *d*, $J = 1.96$ Hz) and one 1,3,4-trisubstituted aromatic ring (6.98, 1H, *d*, $J = 2$ Hz; 7.03, 1H, *d*, $J = 8$ Hz; 7.11, 1H, *dd*, $J = 8, 2$ Hz). The remaining ^1H NMR signals accounted for the methoxyl (δ2.98, 3H, *s*); the oxygenated benzylic methylene (4.13, 2H, *s*) and the three bisbenzylic methylenes (4.01, 4H, *s*; 4.03, 2H, *s*). The fact that the protons on the tetrasubstituted aromatic ring were *meta*-coupled indicated that dimerization had occurred according to Scheme 1. The benzylic position of the methoxy group of rawsonol (**5**) is favoured over the alternate phenolic position because of the known ability of avrainvilleol (**1**) to undergo solvent exchange at the benzylic position to form the methyl ether **2**. In addition, the IR spectrum of the hexa-acetate (**6**) indicates that all the acetate groups are phenolic and the ^1H NMR chemical shifts of the benzylic methylene signals in **6** are similar to those of **5**. The close agreement between calculated [5] and observed ^{13}C NMR chemical shift values for **6** provided confirming evidence for the proposed structure.

It has been proposed that the origin of these diphenylmethanes is from the dimerization of more ubiquitous precursors such as halo-*p*-hydroxylbenzyl alcohol derivatives formed in the shikimic acid pathway via tyrosine [7]. The occurrence of the benzylic alcohol **4** along with **1** and **3** in the same extract of *A. nigricans* supports the proposed mechanism for the formation of **1** and **3** from **4** [4]. The formation of rawsonol (**5**) represents a further condensation by an analogous mechanism (Scheme 1). The propensity for condensation in this class of compounds explains their instability and ease with which they polymerize to insoluble tars.

Assays for inhibition of HMG-CoA reductase activity were performed as previously reported [8, 9] using a purified catalytic fragment of human HMG-CoA reductase [10]. The assay conditions were as reported, except for the concentration of HMG-CoA, NADPH and enzyme which were 10 μM, 200 μM and 20 pM, respectively. Rawsonol (**5**) gave an IC_{50} of 5 μM. For comparison, the fungal metabolite mevinolin, which is the most potent known inhibitor for this enzyme has been shown to have an IC_{50} of 2 nM in this assay. The inhibition observed for rawsonol (**5**) is therefore quite modest, but intriguing because of its structural dissimilarity to known inhibitors of HMG-CoA reductase.



Scheme 1.

EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra were obtained at 270 MHz and 67.8 MHz respectively employing the GASPE technique for carbon spectra with INEPT experiments to distinguish multiplicities. All chemical shifts are reported with respect to TMS (δ0). Fast atom bombardment (FAB) mass spectra were obtained on a VG ZAB-HF mass spectrometer; the sample (*ca* 10 μg) was dispersed on a stainless steel probe tip in a matrix of dithiothreitol/dithioerythritol (3:1). Accurate mass measurements were made at an instrument resolution of 10000 (m/ΔM) by linear voltage scanning. All solvents used were HPLC grade.

Collection and isolation. Avrainvillea rawsoni (specimen No. CI-86-136) was hand-collected using SCUBA (−10 m) at Pte. des Salinas, Martinique, and was frozen within 1 hr of collection. The algae was stored at −30° for 12 months prior to work-up. The sample was lyophillized and the dried algae (48 g) was extracted (3 × 600 ml) with CH_2Cl_2–MeOH (1:1) to give the crude extract (3.1 g, 6.4% dry wt) which was shown to inhibit the action of HMG-CoA reductase. The crude extract was partitioned between H_2O and EtoAc, the organic layer was evapd *in vacuo* and further partitioned between hexane and MeCN. Chromatography of the MeCN layer with MeOH on Sephadex LH-20 (100 g) gave crude rawsonol (**5**). Final purification by reverse phase HPLC on Partisil (Whatman ODS-3, M-9, MeCN–H_2O, 1:1) gave pure rawsonol (**5**, 6.0 mg, 0.01% dry wt), which rapidly darkened on contact with air.

Rawsonol (**5**). IR ν_{max}^{KBr} cm^{-1}: 3000-3600; UV λ_{max}^{MeOH} nm: 285 (ε = 3200), UV $\lambda_{max}^{MeOH+NaOH}$ nm: 300 (ε = 3900); ^1H NMR

($CDCl_3/d_4$-MeOH): δ3.03 (3H, *s*), 3.98 (2H, *s*), 4.01 (2H, *s*), 4.04 (2H, *s*), 4.07 (2H, *s*), 6.06 (1H, *s*, H-5″), 6.85 (1H, *d*, J=2 Hz, H-2‴), 6.86 (1H, *s*, H-5), 6.87 (1H, *d*, J=8 Hz, H-5‴), 7.07 (1H, *dd*, J=2, 8 Hz, H-6‴), 7.24 (1H, *d*, J=1.8 Hz, H-6′), 7.31 (1H, *d*, J=1.8 Hz, H-4′); LR FABMS m/z=800/802/804/806/808.

Rawsonol hexa-acetate (**6**). Rawsonol (**5**, 5.0 mg, 0.006 mmol), was stirred overnight at room temp. in a soln of Ac_2O (1.0 ml) and dry pyridine (1.0 ml). The solvents were removed *in vacuo* and the crude product was chromatographed on silica HPLC (Whatman M9, 3% *i*-PrOH–CH_2Cl_2) to give the pure hexa acetate, **6** (6.0 mg, 0.006 mmol, 90% theoretical), which exhibited the following spectral characteristics: IR ν_{max}^{KBr} cm^{-1}: 1773, 1594, 1574, 1371, 1199, 757; ^1H NMR ($CDCl_3$): δ2.17 (*s*, 3H), 2.26 (*s*, 3H), 2.30 (*s*, 3H), 2.32 (*s*, 6H), 2.33 (*s*, 3H), 2.98 (*s*, 3H), 4.01 (*s*, 4H), 4.03 (*s*, 2H), 4.13 (*s*, 2H), 6.64 (*s*, 1H, H-5″), 6.98 (*d*, 1H, J=2 Hz, H-2‴), 7.00 (*s*, 1H, H-5), 7.03 (*d*, 1H, J=8 Hz, H-5‴), 7.11 (*dd*, 1H, J=8, 2 Hz, H-6‴), 7.26 (*d*, 1H, J=1.96 Hz, H-6′), 7.40 (*d*, 1H, J=1.96 Hz, H-4′); ^{13}C NMR ($CDCl_3$): δ20.1 (*q*), 20.2 (*q*), 20.3 (2C, *q*), 20.7 (2C, *q*), 31.8 (*t*), 32.0 (*t*), 35.8 (*t*), 58.5 (*q*), 68.8 (*t*), 116.0 (*s*), 116.1 (*s*), 116.5 (*s*), 120.7 (*s*), 123.7 (*d*), 123.8 (*d*), 128.1 (*d*), 128.3 (*d*), 129.0 (*d*), 130.7 (*s*), 132.8 (*d*), 133.0 (*d*), 133.2 (*s*), 136.0 (*s*), 136.8 (*s*), 137.3 (*s*), 137.6 (*s*), 138.4 (*s*), 139.8 (*s*), 141.9 (*s*), 142.3 (*s*), 142.9 (*s*), 146.7 (*s*), 147.0 (*s*), 166.8 (*s*), 167.1 (*s*), 167.2 (*s*), 167.8 (*s*), 168.4 (*s*), 168.5 (*s*); high resolution FABMS ($M+Na^+$) found m/z =1076.8815 ($C_{41}H_{36}{}^{79}Br_3{}^{81}Br$, requires 1076.8766).

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