The less polar product [0.02 g (9%); R_f 0.34 (1:4 EtOAc/hexane)] was crystallized from Et_2O and hexane and was identified as the oxazolone **39**: mp 152.5–153.5 °C; IR ($CHCl_3$) 2950, 1825, 1820, 1650, 1585, 1255 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.05 (m, 2), 7.67 (m, 2), 7.41 (m, 6), 5.95 (s, 1), 3.11 (d, 1, J = 12 Hz), 2.50 (d, 1, J = 12 Hz); ^{13}C NMR δ (multiplicity in off resonance) 169.244 (s), 157.195 (s), 135.437, 132.339, 130.432, 128.825, 128.675, 128.448, 127.762, 126.973, 102.736 (s), 64.770 (s), 39.304 (d), 39.278 (t); mass spectrum, m/z (relative intensity) 327, 329 (13, 11), 326, 328 (63, 63), 250 (10), 221 (3), 171, 173, 175 (1:2:1), 120 (2), 105 (96); mass spectrum (FAB), m/z 449, 451, 453 (7, 14, 7), 448, 450, 452 (24, 48, 24). Anal. Calcd for $C_{18}H_{13}O_3NBr_2$: C, 48.10; H, 2.89; N, 3.12; Br, 35.20. Found: C, 47.87; H, 2.80; N, 3.00; Br, 35.64.

Acknowledgment. Support for this research through a grant from the National Institutes of Health (PHS-5-R01-AM27526) is gratefully acknowledged. Carbon-13 nuclear magnetic resonance spectra were obtained at the University of Illinois NSF Regional Instrumentation Facility (Grant No. NSF CHE 79-16100). High-resolution, field-desorption, and fast-atom-bombardment mass spectra were obtained in the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois, supported in part by a grant from the National Institutes of General Medical Sciences (GM 27029). M.J.S. was supported by a fellowship from the University of Illinois and by a National Institutes of Health traineeship.

Registry No. **9**, 6829-40-9; **10**, 96-86-6; **11**, 86766-03-2; **12**, 6813-61-2; **13**, 86748-58-5; **14**, 17585-69-2; **15**, 5874-61-3; **16**, 86748-59-6; **17**, 86748-60-9; **18**, 86748-61-0; **19**, 86748-62-1; **20a**, 86783-91-7; **20b**, 86748-63-2; **21**, 86748-64-3; **22**, 86748-65-4; **23**, 86748-66-5; **24**, 86748-67-6; **25**·HCl, 7524-50-7; **26**, 40216-77-1; **27**, 86748-68-7; **28**, 86748-69-8; **29**, 86748-70-1; **30**, 86748-71-2; **31**, 86748-73-4; **33**, 71849-99-5; **34**, 29670-63-1; **35**, 28687-81-2; **36**, 86766-04-3; **37**, 86748-74-5; **38**, 86748-75-6; **39**, 86748-76-7; benzoyl chloride, 98-88-4; 3-(trimethylsilyl)propargyl bromide, 38002-45-8; *N*-benzoylphenylalanine, 2566-22-5; 2,3-dibromo-1-propene, 513-31-5; benzyl bromide, 100-39-0; benzaldehyde, 100-52-7; hexamethyldisilazane, 999-97-3; protease, 9001-92-7.

Spatane Diterpenoids from the Tropical Marine Algae *Spatoglossum schmittii* and *Spatoglossum howleii* (Dictyotaceae)

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Received September 13, 1982

Three new diterpenoids of the spatane class, spatol (1), the triol **2**, and a triacetate derivative of **3**, have been isolated from the tropical brown algae *Spatoglossum schmittii* and *Spatoglossum howleii*. The diol **4** and the tetraol **5**, metabolites previously isolated from the related alga *Stoechospermum marginatum*, were also components of these algae. Spatol was found to inhibit synchronous cell division in the fertilized sea urchin egg assay and to inhibit human cancer cell cleavage in vitro in the 1–5 μg/mL range.

Preliminary pharmacological investigations of extracts of brown marine algae of the family Dictyotaceae have illustrated considerable antibacterial,[2-9] antiviral,[2,4,9] and cytotoxic activities.[2,10,11] In several subsequent studies the active compounds have been isolated and shown to be novel new diterpenoids or metabolites of mixed biosynthesis.[12-19] Our investigations of this family of marine algae have emphasized the isolation of biologically active metabolites from new genera not yet investigated, and in a recent paper, we described the structure of a new cytotoxic diterpenoid, spatol (1), from extracts of the brown algae *Spatoglossum schmittii*.[19] In this paper we report, in full, on the isolation of spatol and several related spatane diterpenoids from two *Spatoglossum* species, *S. schmittii* and *S. howleii*, both of which are common algae of the Galapagos Islands.

Spatoglossum schmittii Taylor and *S. howleii* (Setchell and Gardner)[20] were found in luxuriant growth along the western coast of Isla Isabella, Galapagos Islands (Archipelago de Colon) in February 1978, and the freshly collected algae were immediately preserved in 2-propanol. Subsequent chloroform-methanol extraction of each species, followed by silica chromatography of the condensed extracts, yielded mixtures rich in the spatane diterpenoids **1–5**. The extract of *S. schmitii* yielded diterpenoids **1**, **2**, and **4**, while the *S. howleii* extract yielded diterpenoids **2–5**. The diterpenoids **1–3** were recognized as new modifications of the spatane ring system, while **4** and **5** were identical with two compounds earlier isolated from the related alga *Stoechospermum marginatum* from the Indian Ocean.[21] Recently, several spatane and sec-

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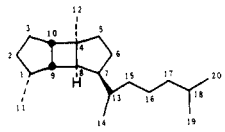
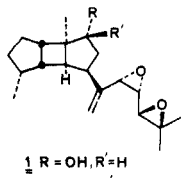
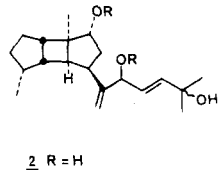
ospatane diterpenoids have also been reported from the Australian alga *Dilophus marginatus*.[22]

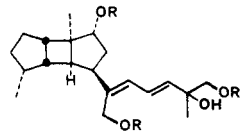




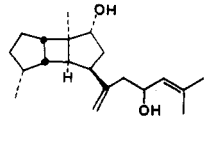
1 R = OH, R' = H
6 R = OAc, R' = H
7 R = R' = O



2 R = H
10 R = Ac



3 R = H
12 R = Ac



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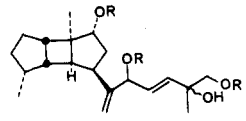
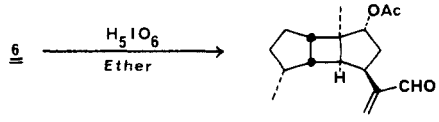


5 R = H
13 R = Ac

Spatol (1), the least polar component of the *S. schmittii* extract, crystallized as fine white needles (mp 100–102 °C) from one HPLC fraction. A molecular formula of $C_{20}H_{30}O_3$ was established for this compound by high-resolution mass spectrometry and by analysis of ^{13}C NMR data (see ref 19). ^{13}C NMR bands at 54.9 (d), 57.1 (d), 58.4 (d), 58.4 (s), and 79.7 (d) indicated the three oxygen atoms in spatol were arranged in two epoxide constellations (one disubstituted and one trisubstituted) and in a secondary alcohol moiety. Consideration of the unsaturation inherent in the molecular formula for 1, eliminating the epoxide and olefin unsaturation, showed spatol to be a tricyclic compound of probable diterpenoid origin. Acetylation of spatol yielded the corresponding acetate 6, which was accompanied by a consistent downfield shift in the ^1H NMR spectum of a doublet (J = 4 Hz) at δ 4.84 in 6. Oxidation of spatol with pyridinium chlorochromate gave ketone 7. On the basis of an infrared absorption at 1740 cm^{-1}, the carbonyl group of 7 was assigned as being in a five-membered ring.

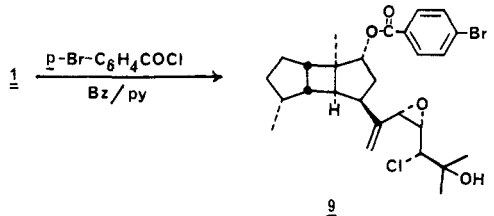
The placement of the epoxides and terminal olefin in spatol, on the side chain at C-13–C-18, was secured by cleavage of the epoxide at C-15–C-16 in spatol acetate (6) with periodic acid. The initial placement of these groups



8

in the side chain was accomplished through a careful analysis of the ^1H NMR data obtained for spatol.[19] The periodic acid cleavage product (8), an α-methylene aldehyde, analyzed for $C_{17}H_{24}O_3$ by mass spectrometry, which indicated the loss of a five-carbon fragment from 6. Elimination of the unsaturation from the aldehyde, acetate, and olefin components of 8 illustrated that the tricyclic nucleus of spatol had been retained in this derivative. Compound 8 possessed two methyl groups, as evidenced by a singlet resonance at δ 0.91 and a doublet band (J = 6 Hz) at δ 0.67. Subtraction of the three-carbon methylene aldehyde unit, two methyl groups, and the acetate from the molecular formula of 8 showed the tricyclic component in this derivative, and hence that of 1, to involve 10 carbon atoms. Since the hydroxyl-bearing center in spatol was assigned to a cyclopentane carbon, the most reasonable tricyclic nucleus that accounted for these data was the tricyclo[5.3.1^{2,6}.0]decane system, as already observed in the bourbonene class of sesquiterpenoids.[23] Several sterochemical features of the structure of spatol were predicted by complete spin-decoupling analysis of the unsaturated aldehyde 8. However, because of the unreliability of coupling constant analyses in five-membered rings, these assignments were considered tenuous.

The complete structure assignment for spatol, including absolute stereochemical designation, was accomplished by a single-crystal X-ray analysis of the *p*-bromobenzoate derivative 9. Reaction of spatol with excess *p*-bromobenzoyl chloride in pyridine produced acylation of the C-5 hydroxyl but also afforded an apparent nucleophilic epoxide displacement (by chloride) at C-17. Assuming inversion at this carbon during the displacement reaction, the epoxide precursor in 1 was assigned the *R* configuration. Details of the full X-ray experiment were provided in an earlier paper.[19] On the basis of these conclusions, spatol (1) can be fully defined, using the semisystematic nomenclature earlier proposed, as 15(*S*),16(*S*):17(*R*),18-diepoxy-5(*R*)-hydroxyspata-13-ene.



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The triol 2 was isolated from more polar chromatography fractions of both algal extracts. The triol, isolated as a crystalline solid, mp 55–57 °C, showed $[\alpha]_D$ −33° (*c* 0.73, $CHCl_3$) and analyzed for $C_{20}H_{32}O_3$ by high-resolution mass spectrometry and ^{13}C NMR. Acetylation under mild conditions (Ac_2O/py/room temperature) yielded a diacetate, 10, which indicated that one of the hydroxyl groups in 2 was tertiary. Two methyl group singlets in the ^1H NMR spectrum at δ 1.30 (6 H, s) illustrated that the tertiary alcohol was positioned on the side chain at C-18. In addition to the typical terminal olefin at C-13–C-14, the triol was found to possess a disubstituted *E* olefin. Proton NMR bands at δ 5.87 (d, J = 16 Hz) and 5.57 (dd, J = 16, 7 Hz), the latter of which was coupled to an α to hydroxyl proton at δ 4.43 (d, J = 7 Hz), indicated an alcohol to be positioned at C-15 with an adjacent *E* olefin at C-16–C-17. The lack of UV absorption for a diene chromophore precluded the alternative structure with hydroxyl positioned at C-17.

Having established the structure of the side chain in the triol 2, the identity of the tricyclic nucleus was confirmed by interrelation of a derivative of 2 with the same deriv-

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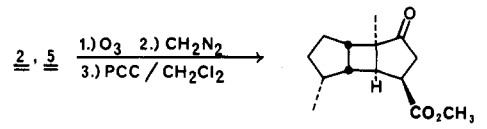
ative from the tetraol **5**. Ozonization of both **2** and **5**, followed by treatment with CH_2N_2 and oxidation of the C-5 hydroxyl with pyridinium chlorochromate, yielded the keto ester **11**, which was identical from both sources. The



11

mutual conversion of **2** and **5** to **11** indicated that each possessed the same tricyclic nucleus with identical absolute stereochemistries at six common centers. As reported earlier,[21] the structure of the tetraol **5** had been proven by relating it to spatol via its conversion to the unsaturated aldehyde **8**. The stereochemistry of the hydroxyl at C-5 in **2** was assigned as *R* on the basis of its ^1H NMR features, which were identical with those of **1** and several other spatane diterpenoids. The 5*S* alcohol configuration, as encountered in other synthetic derivatives, yields different coupling constants and chemical shifts for the C-5 proton.[21] Thus, triol **2** was confidently assigned as 5(*R*)-15,18-trihydroxyspata-13,16(*E*)-diene. No data could be interpreted to assign the alcohol stereochemistry at C-15.

The very polar fractions from the silica gel column chromatography of the *S. howleii* extract (eluted with 100% MeOH) were recognized to possess a polar tetraol conceived as **3**. Isolation and successful purification of this compound could only be achieved, however, by acetylation (Ac_2O/py/room temperature) of the fraction and purification of the acetate mixture by HPLC. A major product isolated was the triacetate alcohol **12**, which showed $[\alpha]_D$ +42.7° and analyzed for $C_{26}H_{38}O_7$ by combined mass spectral and ^{13}C NMR off-resonance techniques. Comparison of the ^1H NMR features of **12** with those of the crude, unacetylated fraction showed the expected shifts of the protons at C-5, C-14, and C-20 (resulting from acetylation), further substantiating **3** as the nonmodified natural product. Comparison of the ^1H and ^{13}C NMR bands recorded for the triacetate **12** with those of numerous spatane diterpenoids indicated that in **12** the tricyclic spatane skeleton remained intact. Analysis of ^1H NMR features for the C-5 proton further showed that the acetate had an identical stereochemistry as in **1**, **2**, **4**, and **5**. All modifications were, therefore, conceived to be located on the side chain. Most conspicuous were the absence of the spectral features associated with the terminal olefin at C-13–C-14. These bands were replaced by those assigned to a conjugated diene system that was also observed to produce UV absorption at 236 nm (ϵ 16 500). The diene was placed between C-13–C-15 and C-16–C-17 on the basis of an unmeasurable, but clear, allylic coupling between the C-7 proton at δ 3.32 (m) and the olefinic broadened doublet at δ 6.16 (J = 10 Hz). The disubstituted olefin was assigned the *E* configuration on the basis of the observed vicinal coupling of 15 Hz. Another spectral feature of the side chain was the existence of two AB double doublets at δ 4.10 and 3.96 (J = 9 Hz) and δ 4.68 and 4.50 (J = 11 Hz). These bands, and the absence of two of the three side-chain methyls, indicated that the two methyls had been converted to primary acetate functionalities. Finally, the stereochemistry of the C-13–C-15 olefin was assigned as *Z* on the basis of the absence of NOE enhancement recorded when the C-14 methylene was strongly irradiated. On the basis of these deductions, the triacetate alcohol **12** was defined as 18-hydroxy-5,14,19-triacetoxyspata-13(15)(*E*),16(*Z*)-diene.

Two previously described metabolites, the diol **4** and the tetraol **5**, were also isolated in this investigation. The diol **4** was isolated as a major component of both *Spatoglossum* species, while the tetraol **5**, isolated as the triacetate **13**, was a major metabolite only of *S. howleii*. Both compounds were identical with metabolites isolated or synthesized earlier from *Stoechospermum marginatum*, as determined by detailed spectral comparisons.

Spatol (**1**), the diol **4** and the tetraol **5** all showed cytotoxic effects against the fertilized egg of the Pacific sea urchin *Lytechinus pictus*, albeit at different levels. Spatol showed ED_{50} values of 1.2 μg/mL in this assay and was also cytotoxic toward human T242 melanoma and 224C astrocytoma neoplastic cell lines in in vitro culture. In contrast, the diol **4** and tetraol **5** showed ED_{50} values of 16 μg/mL in the urchin egg assay. Further, spatol was shown to be lethal, at 100 μg/mL, to the common goldfish *Carassius carassius* and to inhibit growth, at 100 μg/mL, of the marine diatom *Phaeodactylum tricornutum*.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer Model 137 spectrophotometer and ultraviolet spectra were recorded on a Beckman MVI spectrophotometer. Optical rotations were recorded on a Perkin-Elmer Model 141 polarimeter, using a 10-cm microcell. ^1H NMR spectra were recorded on homebuilt 360-MHz (Oxford Magnetics, Nicolet computer), Varian HR-220, and Varian T-60 NMR spectrometers; chemical shifts are reported relative to Me_4Si (δ 0), and coupling constants are given in hertz. ^{13}C NMR spectra were recorded on Varian CFT-20, and Nicolet 200-MHz wide-bore spectrometers. Low-resolution mass spectra were obtained from a Hewlett-Packard 5930-A mass spectrometer and high-resolution mass spectra were supplied by the Department of Chemistry, University of California, Los Angeles, and the Mass Spectrometer Facility at Colorado State University. Melting points were determined on a Fisher-Johns melting point apparatus and are reported uncorrected. All solvents used were distilled from glass prior to use.

Algal Collection and Isolation of Spatane Diterpenoids. *Spatoglossum schmitii* and *Spatoglossum howleii* were collected in February 1978, by hand, using SCUBA (–16 to –20 m), at Punta Vicente Roca, Isla Isabella and Punta Espanosa, Isla Fernindina, Archipelago de Colon (Galapagos Islands).[20] The collections were separated and stored in isopropyl alcohol, and within 3 weeks, the IPA was decanted and the algae homogenized and repeatedly extracted with warm $CHCl_3$/MeOH (2:1). The $CHCl_3$/MeOH was removed in vacuo and the residue partitioned between saturated brine (0.5 L) and $CHCl_3$ (4 × 0.5 L). The $CHCl_3$ extracts were dried over $MgSO_4$, and the solvent was evaporated to yield 10.7 g of extract for *S. schmittii* and 12.4 g of extract for *S. howleii*, both as greenish-brown tars (dry weight of extracted algae 0.387 and 0.435 kg, respectively). Each extract was applied separately to a column (6.5 × 100 cm) of Davison Grade 62 silica gel, and fractions were eluted wiih solvents of increasing polarity (petroleum ether/dichloromethane/ethyl acetate/methanol).

Relatively nonpolar fractions of the *S. schmittii* extract (10% EtOAc/CH_2Cl_2) contained **1**, which was further purified by using HPLC (μ-Porasil, 50% EtOAc/isooctane). Compound **4** eluted next (20% EtOAc/CH_2Cl_2) and was also further purified by HPLC (μ-Porasil, 50% EtOAc/isooctane). From more polar fractions (100% EtOAc) the triol **2** was also isolated by HPLC (μ-Porasil, 70% EtOAc/isooctane).

From the *S. howleii* extract the diol **4** eluted first (20% EtOAc/CH_2Cl_2) and the triol **2** second (100% EtOAc). Each were purified by using the same HPLC conditions employed above. The very polar fractions (100% MeOH) were acetylated with excess acetic anhydride in pyridine at 25 °C, and the acetate mixture was rechromatographed over a small column (1.0 × 50 cm) of Davison Grade 62 silica gel to sequentially yield **9** and **12** (20% EtOAc/CH_2Cl_2). Both **9** and **12** were further purified by HPLC (μ-Porasil, 50% EtOAc/isooctane).

Acetylations. All acetylations were conducted in a similar fashion. The natural product (10–20 mg) was combined with excess pyridine (2 mL) and Ac_2O (2 mL) with stirring at 0 °C. The reaction was allowed to warm to room temperature, and after

16–24 h, ice then water were added, and the mixture was extracted with Et_2O (3 × 25 mL). The combined Et_2O extracts were washed first with 5% HCl (3 × 25 mL) and next with saturated $NaHCO_3$ solution (3 × 25 mL). The Et_2O phase was dried over anhydrous $MgSO_4$ and reduced in vacuo to yield the acetylated products. Purifications, when necessary, were performed with silica HPLC.

Spatol (1, 15(*S*),16(*S*):17(*R*),18-Diepoxy-5(*R*)-hydroxyspata-13-ene). Spatol crystallized from warm petroleum ether as fine white needles, mp 100–102 °C, and composed 2.7% of the extract of *S. schmittii*. Spatol possessed the following spectral features: $[\alpha]_D$ +45.6° (*c* = 1.56, $CHCl_3$); IR (film) 3400, 2950, 1640, 1450, 1380, 1250, 1180, 1120, 1050, 945, 910, 840 cm^{-1}; MS, M^+ *m/z* 318 amu; ^1H and ^{13}C NMR data were reported earlier.[19] Spatol acetate (6): IR (CCl_4) 2950, 1745, 1460, 1380, 1245, 1185, 1110, 1030, 915, 840 cm^{-1}; ^1H NMR (220 MHz, CCl_4) δ 5.11 (1 H, d, *J* = 0.9 Hz), 5.00 (1 H, s), 4.84 (1 H, d, *J* = 4 Hz), 3.32 (1 H, dd, *J* = 0.8, 4 Hz), 2.88 (1 H, m), 2.70 (1 H, dd, *J* = 4.0, 8.5 Hz), 2.32 (1 H, d, *J* = 8.5 Hz), 2.32 (1 H, ddd, *J* = 13, 13, 4 Hz), 2.14 (3 H, m), 2.02 (3 H, s), 1,2–2.0 (6 H, m), 1.41 (3 H, s), 1.30 (3 H, s), 0.95 (3 H, d, *J* = 6 Hz), 0.89 (3 H, s).

Spatol Ketone (7). Excess pyridinium chlorochromate was added to a stirred solution of spatol (1; 29 mg, 0.091 mmol) in CH_2Cl_2 (5 mL) with excess sodium acetate buffer at 0 °C. The reaction was allowed to warm to room temperature and in 1.5 h was complete as determined by TLC. The reaction mixture was diluted with diethyl ether (50 mL) and filtered through a thin layer of silica gel, and the solvents were removed in vacuo to give a high yield of the oily ketone 7 (28 mg, 0.008 mmol, 97%), which was pure by TLC and ^1H NMR analyses. Ketone 7 showed the following spectral characteristics: IR ($CHCl_3$) 2950, 1740, 1450, 1370, 1215, 1090, 1040, 940, 910, 820 cm^{-1}; ^1H NMR (220 MHz, $CDCl_3$) δ 5.30 (1 H, br s), 5.15 (1 H, s), 3.55 (1 H, br d, *J* = 4 Hz), 2.98 (2 H, m), 2.91 (1 H, dd, *J* = 4, 8.5 Hz), 2.48 (3 H, m), 2.40 (2 H, m), 1.91 (1 H, m), 1.75 (3 H, m), 1.59 (1 H, m), 1.45 (3 H, s), 1.34 (3 H, s), 0.97 (3 H, s), 0.97 (3 H, d, *J* = 6 Hz).

***p*-Bromobenzoate Derivative 9.** To 19 mg of spatol (1; 0.060 mmol) in benzene (1 mL) and pyridine (1 mL) was added with stirring an excess of *p*-bromobenzoyl chloride, dissolved in benzene and pyridine (ca. 1 mL each). The reaction was heated to 57 °C and run overnight, quenched with ice water, and extracted with diethyl ether (3 × 25 mL), which in turn was extracted with 5% HCl (3 × 25 mL) and saturated $NaHCO_3$ solution (3 × 25 mL). The ether was evaporated to yield a solid residue that was composed of several UV-absorbing compounds by TLC. The mixture was separated first by preparative thick-layer chromatography and then by HPLC of the highest R_f band (μ-Porasil, 10% EtOAc/isooctane) to yield pure 9 as a solid (12 mg, 0.022 mM, 37%), which displayed the following ^1H MMR spectral bands: (220 MHz, $CDCl_3$) δ 7.82 (2 H, d, *J* = 7 Hz), 7.54 (2 H, d, *J* = 7 Hz), 5.20 (1 H, s), 5.16 (1 H, d, *J* = 4 Hz), 5.12 (1 H, s), 3.68 (1 H, d, *J* = 10 Hz), 3.59 (1 H, d, *J* = 4 Hz), 3.38 (1 H, dd, *J* = 4, 10 Hz), 2.97 (1 H, m), 2.50 (1 H, ddd, *J* = 4, 13, 13 Hz) 2.19 (3 H, m), 1.2–2.0 (8 H, m), 1.27 (3 H, s), 1.23 (3 H, s), 0.98 (3 H, s), 0.98 (3 H, s), 0.89 (3 H, d, *J* = 6 Hz).

Triol 2, 5(*R*),15,18-Trihydroxyspata-13,16(*E*)-diene. The triol 2 was isolated as a crystalline solid, mp 55–57 °C, as 3.5% and 3.2% of the extracts of *S. schmittii* and *S howleii*, respectively. Triol 2 illustrated the following spectral features: $[\alpha]_D$ −33.4° (*c* 0.73, $CHCl_3$); IR ($CHCl_3$) 3450, 2950, 1640, 1450, 1425, 1235, 1110, 1040, 970, 910 cm^{-1}; HRMS (70 eV), *m/z* 287.2018 (M^+–CH_3H_2O, 0.7 mamu deviation, $C_{19}H_{27}O_2$, 5.2), 215.1425 ($C_{15}H_{19}O$, 13.1), 202.1345 ($C_{14}H_{18}O_1$, 16.4), 187.1503 ($C_{14}H_{19}$, 13.8), 167.1096 ($C_{10}H_{15}O_2$, 20.3), 159.1201 ($C_{12}H_{15}$, 34), 135.1171 ($C_{10}H_{15}$, 67), 93.0693 (C_7H_9, 68), 81.0705 (C_6H_9, 100); low-resolution mass spectrum (70 eV, 200 °C); obsd $M^+ - H_2O$ *m/z* 302 (>0.1), 287 (0.10); ^1H NMR (220 MHz, $CDCl_3$) δ 3.66 (1 H, d, *J* = 4 Hz, H-5), 2.25 (1 H, ddd, *J* = 13, 13, 4 Hz, H-6β), 2.00 (1 H, m, H-6α), 2.84 (1 H, m, H-7), 0.88 (3 H, d, *J* = 6 Hz, H-11), 0.96 (3 H, s, H-12), 5.32 (1 H, br s, H-14), 4.96 (1 H, br s, H-14), 4.43 (1 H, d, *J* = 7 Hz, H-15), 5.57 (1 H, dd, *J* = 16, 7 Hz, H-16), 5.87 (1 H, d, *J* = 16 Hz, H-17), 1.30 (6 H, s, H-19, 20); ^{13}C NMR (20 MHz, $CDCl_3$) δ 149.7 (s), 140.3 (d), 128.2 (d), 108.3 (t), 79.8 (d), 74.4 (d), 70.3 (s), 46.9 (s), 43.2 (d), 42.9 (d), 42.4 (d), 37.4 (d), 36.9 (d), 36.4 (t), 35.1 (t), 29.5 (q), 29.2 (q), 27.9 (t), 14.6 (q), 14.3 (q). Triol diacetate 10: IR ($CHCl_3$) 3600, 3000, 1730, 1640, 1450, 1410, 1330, 1020, 895 cm^{-1}; ^1H NMR (220 MHz, $CDCl_3$) δ 5.91 (1 H, d, *J* = 16 Hz), 5.52 (2 H, m), 5.18 (1 H, s), 4.91 (1 H, s), 4.86 (1 H, d, *J* = 4 Hz), 2.80 (1 H, m), 2.29 (1 H, dd, *J* = 4, 13, 13 Hz), 2.07 (3 H, s), 2.00 (3 H, s), 1.2–2.1 (10 H, m), 1.32 (6 H, s), 0.91 (3 H, d, *J* = 6 Hz), 0.86 (3 H, s).

Ozonolysis, Methylation, and Oxidation of Triol 2 To Yield Keto Ester 11. Ozone was bubbled through a solution of EtOAc (10 mL) containing 110.7 mg of 2 (0.356 mmol) at −78 °C for 0.6 h. Excess ozone was next removed by purging with argon. The reaction mixture was treated with an excess of CH_2N_2 in Et_2O, and after several hours the reactants were allowed to evaporate in the hood. Chromatography of the crude residue over silica gel eluting with 15% EtOAc/CH_2Cl_2 yielded the C-5 alcohol–methyl ester (9.0 mg, 10.6%), which showed the following spectral features: $[\alpha]_D$ +4.2° (*c* 0.90, $CHCl_3$); ^1H NMR (220 MHz, $CDCl_3$) δ 3.84 (1 H, d, *J* = 4 Hz), 3.61 (3 H, s), 3.23 (1 H, ddd, *J* = 6.5, 6.5, 13 Hz), 2.52 (1 H, dd d, *J* = 4, 13, 13 Hz), 2.23 (1 H, m), 1.0–1.1 (9 H, m), 0.98 (3 H, s), 0.89 (3 H, d, *J* = 6 Hz). The purified alcohol (7.4 mg) was added to a stirred CH_2Cl_2 solution of excess pyridinium chlorochromate in the presence of sodium acetate buffer at 0 °C for 1.8 h. The reaction mixture was diluted with 25 mL of diethyl ether and filtered through a thin layer of silica gel, and the solvents were evaporated in vacuo. The resulting keto ester 11 was purified by HPLC (μ-Porasil, 20% EtOAc/isooctane; 4.7 mg yield, 0.020 mmol, 64.6%) and showed $[\alpha]_D$ −185° (*c* 0.47, $CHCl_3$): IR ($CHCl_3$) 2950, 1735, 1705, 1460, 1440, 1370, 1230, 1175, 1025, 940 cm^{-1}; ^1H NMR (220 MHz, $CDCl_3$) δ 3.73 (3 H, s), 3.18 (2 H, m), 2.54 (2 H, m), 2.36 (1 H, dd, *J* = 7.5, 7.5 Hz), 1.0–2.0 (6 H, m), 0.93 (3 H, s), 0.90 (3 H, d, *J* = 6 Hz).

Ozonolysis, Methylation, and Oxidation of Tetraol 5 To Yield Keto Ester 11. Compound 5 (330 mg, 0.98 mM) in 20 mL of EtOAc was treated with O_3 (35 min, −78 °C) at which time the solution became a light purple color. Excess ozone was removed by bubbling argon through the solution and the EtOAc was removed in vacuo at 10 °C. The resultant oil was dissolved in 90% formic acid (9 mL) and 30% hydrogen peroxide (4.5 mL) at room temperature with stirring. The reaction was slowly heated to 100 °C and maintained at this temperature for 1.2 h and then cooled and quenched with ice. The ether extract (3 × 50 mL) was washed with water (3 × 30 mL), dried over anhydrous $MgSO_4$, and filtered, and the solvents removed in vacuo. The crude reaction product was treated with excess ethereal CH_2N_2 for 1 h. The mixture was crudely fractionated over a small silica gel column (1.0 × 25 cm). Mid-polarity fractions contained the C-5 alcohol derivative, which was further purified by high-performance LC (μ-Porasil, 20% EtOAc/isooctane) to yield 19.7 mg of pure alcohol (0.083 mM, 8.5%).

The alcohol (12 mg, 0.051 mM) was oxidized with pyridinium chlorochromate in NaOAc buffer solution as described above for the conversions of the triol 2. The keto ester 11 was purified by HPLC as above (yield 10.8 mg, 89%) and showed $[\alpha]_D$−226° (*c* 1.06, $CHCl_3$). The NMR features of 11 produced from 5 were superimposable with those of 11 from triol 2.

Triacetate Derivative 12 of Tetraol 3, 18-Hydroxy-5,14,19-triacetoxyspata-13(15)(*E*),16(*Z*)-diene. Acetylation of the 100% MeOH-eluted column fractions from *S. howleii* followed by μ-Porasil HPLC yielded the triacetate 12 as 0.15% of the extract. Attempts to isolate the free tetraol, 3 by chromatographic techniques were unsuccessful due to the extreme "tailing" characteristics of this compound. The triacetate 12 showed the following spectral features: $[\alpha]_D$ +42.7° (*c* = 0.98, $CHCl_3$); IR ($CHCl_3$) 3500, 2950, 1710, 1450, 1360, 1220, 1025, 995, 910 cm^{-1}; UV (MeOH) 236 nm (ε 16 500); HRMS M^+ – HOAc *m/z* 402.2399 for $C_{24}H_{37}O_5$ (0.7 mamu deviation from calcd); ^1H NMR (360 MHz, $CDCl_3$) δ 4.96 (1 H, d, *J* = 4 Hz, H-5), 2.36 (1 H, ddd, *J* = 13, 13, 4 Hz, H-6β), 1.98 (1 H, m, *J* = 13, 6 Hz, H-6α), 3.32 (1 H, m, H-7), 0.72 (3 H, d, *J* = 6 Hz, H-11), 0.88 (3 H, s, H-12), 4.10 (1 H, d, *J* = 9 Hz, H-14), 3.96 (1 H, d, *J* = 9 Hz, H-14), 6.16 (1 H, d, *J* = 10 Hz, H-15), 6.64 (1 H, dd, *J* = 10, 15 Hz, H-16), 5.75 (1 H, d, *J* = 15 Hz, H-17), 4.68 (1H, d, *J* = 11 Hz, H-19), 4.50 (1 H, d, *J* = 11 Hz, H-19), 1.30 (3 H, s, H-20), 2.08 (3 H, s, OAc), 2.06 (3 H, s, OAc), 2.05 (3 H, s, OAc); ^{13}C NMR (50 MHz, $CDCl_3$)[24] δ 170.6 (C), 170.6 (C), 170.6 (C), 138.0 (CH), 134.9 (C),

(24) The protons bonded to carbon in this experiment were determined by polarization transfer spectroscopy; see: Doddrell, D. M.; Tegg, D. T. *J. Am. Chem. Soc.* **1980**, *102*, 6388.

130.4 (CH), 124.8 (CH), 82.1 (CH), 70.9 (CH_2), 68.4 (CH_2), 65.9 (C), 47.0 (CH), 45.8 (C), 43.2 (CH), 41.2 (CH), 39.3 (CH), 36.5 (CH), 34.6 (CH_2), 34.4 (CH_2), 28.0 (CH_2), 25.1 (CH_3), 21.3 (CH_3), 21.1 (CH_3), 20.9 (CH_3), 15.3 (CH_3), 13.1 (CH_3).

Diol 4, 5(*R*),16-Dihydroxyspata-13,17-diene. The diol 4 was isolated as an oil as 8.8% and 7.7% from *S. schmittii* and *S. howleii*, respectively. Diol 4 from the former source showed $[\alpha]_D$ +8.2° (*c* 1.05, $CHCl_3$), while 4 from the latter showed $[\alpha]_D$ + 7.3° (*c* 3.9, $CHCl_3$). The diol as isolated from *Steochospermum marginatum* showed $[\alpha]_D$ +6.3 (*c* 1.91, $CHCl_3$). The spectral features of the diols from *Spatoglossum* were superimposable with an authentic sample from *S. marginatum*.[21]

Triacetate Derivative 13 of Tetraol 5, 18-Hydroxy-5(*R*),15,19-triacetoxyspata-13,16(*E*)-diene. The triacetate derivative, 13 of the tetraol 5 was isolated as 1.0% of the *S. howleii* extract. Compound 13 showed $[\alpha]_D$ −51.2° (*c* 1.16, $CHCl_3$) in close comparison to the synthetic triacetate produced from the tetraol as isolated from *Stoechospermum marginatum*, $[\alpha]_D$ −36° (*c* 1.32, $CHCl_3$).[21] The spectral features of 13 were identical with those of the authentic derivative reported earlier.[21]

Acknowledgment. We thank Dr. James Norris, Smithsonian Institute, for the kind invitation to participate on a marine floristic research investigation in the Galapagos Islands. We gratefully acknowledge the help of Dr. John Wright, Department of Chemistry, Niversity of California, San Diego, in obtaining our high-field NMR data and Dr. Robert Jacobs, University of California, Santa Barbara, for the human cancer cell bioassays. We appreciate the support of the Mass Spectrometry Resource Center, University of California, in providing high-resolution mass spectral analyses. The Center is supported by NIH RRO1614 (A. L. Burlingame, PI). This research is the result of generous financial support from the Sea Grant Program, Department of Commerce, under Grant 04-8-MO1-189. The U.S. Government is authorized to reproduce and distribute reprints notwithstanding any copyright notation that may appear hereon.

Registry No. 1, 76520-52-0; 2, 86887-32-3; 3, 86887-34-5; 4, 77136-83-5; 5, 77136-60-8; 6, 77136-71-1; 7, 86887-30-1; 8, 77136-72-2; 9, 86887-31-2; 10, 86887-33-4; 11, 77136-70-0; 12, 86900-72-3.

Kinetics and Mechanism of the Oxidation of Alcohols by *N*-Bromoacetamide in Alkaline Solution

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Received October 28, 1982

The kinetics of the oxidation of seven secondary alcohols by *N*-bromoacetamide has been studied in alkaline solution. The main product of the oxidation is the corresponding ketone. The reaction is first order with respect to the oxidant and alcohol. The oxidation of benzhydrol-α-*d* indicates the absence of a primary kinetic isotope effect. The rate decreases with the increase in the concentration of hydroxide ion. Addition of acetamide decreases the reaction rate. The rates were determined at four different temperatures, and the activation parameters were evaluated. The activation enthalpies and entropies of the oxidation of the seven alcohols are linearly related. Hypobromite ion has been postulated as the reactive oxidizing species. A mechanism involving rate-determining nucleophilic attack of hypobromite ion on the alcohol molecule has been proposed.

Although many reports about the mechanism of the oxidations by *N*-halo amides like *N*-bromosuccinimide[1] and *N*-bromoacetamide[2] (NBA) in acid solution are available, there seems to be no report about the mechanism in alkaline solution. It is known, however, that the mechanisms of several redox reactions change with the changes in the reaction conditions, e.g., reactions of chloramine-T[3] and permanganate ion.[4] We now report the kinetics of the oxidation of several secondary alcohols by NBA in aqueous alkaline solution and discuss the mechanistic conclusions. For the purpose of comparison, the oxidation of some of the alcohols by hypobromite ion was also studied.

Experimental Section

Materials. All alcohols were commercial products (Fluka). They were dried over anhydrous magnesium sulfate and then fractionally distilled. Benzhydrol-α-*d*[5] and NBA[6] were prepared by reported methods. The isotopic purity of benzhydrol-α-*d* as ascertained by its NMR spectra was 94 ± 4%. While the effect of varying the concentration of sodium hydroxide on the reaction rate was studied, the ionic strength was kept constant at 0.20 M by using sodium perchlorate. Hypobromous acid was freshly prepared by the action of bromine on yellow mercuric oxide.[7] It was then neturalized by sodium hydroxide.

Product Analysis. In a typical experiment propan-2-ol (6.00 g, 0.1 mol), NBA (2.79 g, 0.02 mol), and sodium hydroxide (0.40 g, 0.01 mol) were made up to 100 mL in water. The mixture was kept for ca. 20 h in the dark to ensure completion of the reaction. It was then treated overnight with an excess (200 mL) of a saturated solution of 2,4-dinitrophenylhydrazine in 3 M HCl. The precipitated 2,4-dinitrophenylhydrazone (DNP) was filtered off, dried, weighed, recrystallized from ethanol, and weighed again. The product was identical (melting point and mixture melting point) with an authentic sample of the DNP of acetone. The yields of DNP before and after recrystallization were 4.0 (84%) and 3.5

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