chromatographic analysis and nmr spectrum to be composed of α-chlorostyrene (*ca.* 16%), acetophenone (43%), and phenylacetonitrile (17%), corresponding to maximum yields of *ca.* 7, 19, and 7%, respectively. Each component was identified by its identical comparison with vpc retention time and nmr spectra for authentic samples.

The water layer was made basic with a large excess of sodium carbonate and extracted with ether to give 0.5 g of a dark oil after combining, drying over potassium carbonate, and evaporating the organic layers. Treatment of this oil with anhyrous hydrogen chloride gave a few milligrams of a yellow solid which was shown to be 2,5-diphenylpyrazine, mp 192–194°, by mixture melting point and infrared spectrum.

Reaction of α-Chlorostyrene with Sulfuric Acid.—A solution of 1.7 g (0.012 mol) of α-chlorostyrene in 6 ml of sulfuric acid and 16 ml of absolute ethanol was heated under reflux for 4 hr and allowed to stand overnight at room temperature before being poured into 200 ml of ice water. The mixture was extracted with ether and the combined extracts were dried over potassium carbonate and evaporated to give 1.1 g (73%) of acetophenone as a dark oil. This assignment was verified by infrared spectrum and vpc retention time.

Registry No.—1-Phenyl-2-azido-1-ethanol, 18756-01-9; 1-chloro-1-phenyl-2-azidoethane, 18756-02-0; **5**, 18756-03-1; 1-(β-styryl)-4-phenyl-1,2,3-triazole, 18756-04-2; α-chlorostyrene, 1018-34-8.

Chemistry of Coelenterates. XII.[1a] Hydroxyancepsenolide, a Dilactone from the Octocoral, *Pterogorgia anceps*

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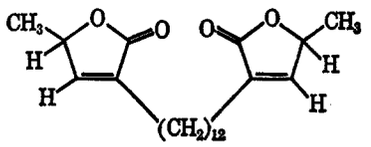
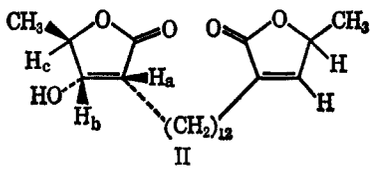
Received August 26, 1968

In an earlier paper,[2] we described the structure elucidation of ancepsenolide, I, a bisbutenolide isolated from the gorgonian, *Pterogorgia anceps* (Pallas).[3] Another dilactone has been isolated from this same organism and in this paper we wish to present evidence which confirms its structure as that shown in II. The new lactone, hydroxyancepsenolide, was isolated by hexane extraction of the dried animal and purified by column chromatography to give a white solid, mp 122.5–123.7°. Mass spectral analysis (*m/e* 380) and combustion data established the molecular formula as $C_{22}H_{36}O_5$. The significant features of the infrared spectrum were a very weak absorption at 3400 cm^{-1} (KBr, hydroxyl) and a strong, broad carbonyl absorption having a maximum at 1750 cm^{-1}. The strong carbonyl absorption at 1750 cm^{-1}, an absorption maximum in the ultraviolet spectrum at 209 mμ with approximately one-half the extinction coefficient (15,800) found for ancepsenolide (28,000), and one-proton multiplets in the nmr spectrum at δ 5.0 and 7.0 ppm identical with ones occurring in the spectrum of ancepsenolide[2] confirmed the presence in hydroxyancepsenolide of one substituted butenolide ring identical with those present in ancepsenolide. The presence of a long methylene chain in hydroxyancepsenolide is indicated by the large absorption peak at δ 1.27 ppm and a long series of peaks in the mass spectrum differing by 14 mass units.

The presence of a hydroxyl group in II was confirmed by the formation of a monoacetate and the secondary nature of this alcohol was inferred from a shift in the nmr spectrum of a one-proton signal (double doublet) centered at δ 4.24 in hydroxyancepsenolide to 5.18 ppm in the corresponding monoacetate.



I



II

Dehydration of II with phosphorus oxychloride in pyridine gave ancepsenolide in good yield. This fact, along with the evidence for the secondary character of the alcohol group, requires that the hydroxyl group of II must be attached to the β carbon of the second five-membered lactone ring. The broad carbonyl absorption in II (1730–1780 cm at one-half peak intensity) is consistent with the presence of both a saturated and an α,β-unsaturated γ-lactone. Hydrogenation of II resulted in the uptake of slightly more than 1 mol of hydrogen and gave a dihydro derivative whose infrared spectrum showed a strong absorption with a maximum at 1765 cm^{-1} (saturated γ-lactone). The nmr spectrum of dihydrohydroxyancepsenolide lacked any vinyl proton absorption and exhibited a complex absorption envelope extending from δ 4.1 to 4.9 ppm which is attributed to a combination of the absorptions due to the three protons attached to carbons bearing oxygen atoms.

The relative stereochemical assignments indicated in structure II for the substituents in the hydroxylated lactone ring are based on coupling-constant data. Proton c of the saturated γ-lactone ring in II appears as a broadened quartet in which the coupling to the methyl group is large, J = 6 cps, and the second splitting attributed to coupling with proton b is small, $J \cong 0.5$–1 cps. The signal assigned to proton b appears as a double doublet in which the small coupling constant, $J \sim 0.5$–1 cps, is consistent with a J_{bc} assignment and the larger J value, 6 cps, must be due to coupling with proton a of the lactone ring. A *trans* orientation of protons b and c imposes a dihedral angle of 105–115° between these protons, and this is consistent with the smaller coupling constant, $J_{bc} \sim 1$ cps.[4] A dihedral angle of close to 0° would be expected between protons a and b if they

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(2) F. J. Schmitz, K. W. Kraus, L. S. Ciereszko, D. H. Sifford, and A. J. Weinheimer, *Tetrahedron Lett.*, 97 (1966).

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(4) For a leading reference, see R. H. Bible, "Interpretation of NMR Spectra," Plenum Press, New York, N. Y., 1965, pp 35–37.

are *cis* to one another and this is consistent with the larger coupling constant assigned to J_{ab}.[4]

In the nmr spectrum of II obtained in deuteriochloroform the absorptions due to the methyl groups are partially masked by the large methylene peak. The lactone methyl absorptions in the acetate of II are shifted upfield to 1.05 ppm (superimposed doublets) when benzene is used as solvent and the integral and J values (6 cps) are easily discernible. In the nmr spectrum of the acetate of dihydrohydroxyancepsenolide obtained in benzene the methyl absorptions appear as doublets centered at δ 0.9 and 1.0 ppm with overlapping peaks at 0.95 ppm.

Experimental Section

Melting points were determined in capillary tubes with a Thomas–Hoover melting apparatus and are corrected. Ultraviolet spectra were measured in 95% ethanol on a Beckman DK-1 spectrophotometer and infrared spectra were taken with a Beckman IR-8 spectrophotometer. Nmr spectra were determined using tetramethylsilane as an internal standard with a Varian A-60 spectrometer.

Isolation of Hydroxyancepsenolide.—Collections of *Pterogorgia anceps* were made along the outside of Boca Chita Key, Miami, Fla., and in Bimini, Bahamas. The air-dried ground gorgonian material (1.9 kg) was extracted consecutively in a continuous percolator–extractor[5] with the following solvents: (1) hexane, 18 hr; (2) hexane, 96 hr; (3) hexane, 48 hr; (4) benzene, 48 hr; (5) benzene, 72 hr; (6) methanol, 28 hr; (7) methanol, 48 hr. The first hexane extract consisted of a complex lipid mixture from which ancepsenolide is isolated by chromatography over alumina[6] or silicic acid.[2] Some hydroxyancepsenolide precipitated from the second hexane extract which contained a total of 1.87 g of material. A 2.84-g sample of crude hydroxyancepsenolide was absorbed on silica gel (140 g, 35 × 653 mm) and eluted with 25% ethyl acetate in benzene (75-ml fractions). Fractions 5–18 contained 1.53 g of white solid which was recrystallized several times from isopropyl alcohol to give white platelets: mp 122.5–123.7°; $[\alpha]^{24}D$ +3.4°;[7] uv max (95% C_2H_5OH) 209 mμ (ϵ 15,800); ir ($CHCl_3$) 3600 very weak (OH), and 1750, broad (lactones); ir (KBr, concentrated) 3600 (OH), 1760, 1720 (lactone C=O's); nmr ($CDCl_3$) δ 7.0 (q, 1, vinyl hydrogen), 5.0 (complex quartet, 1, $CH_3CH(-O^-)CH=$), 4.30–4.73 (broadened q, 1, $-CH(-O^-)CHOH-$), 4.24, (dd, 1, $-CH(-O^-)CHOH-$), 1.1–3.0 ppm (31, $-(CH_2)_{12}-$, $2CH(-O^-)CH_3$, -OH).

Hydroxyancepsenolide Acetate.—Hydroxyancepsenolide (0.266 g, 0.77 mmol) was dissolved in a mixture of 10 ml of pyridine and 1 ml of acetic anhydride and the resulting solution was stirred overnight at room temperature. The reaction mixture was poured into ice water and the product was recovered by extraction into ether. The ether solution was washed with dilute hydrochloric acid, sodium bicarbonate, and water and dried ($MgSO_4$). Evaporation of the ether left 0.288 g of white solid of which 0.180 g was recrystallized four times from isopropyl alcohol, 53 mg, mp 68.3–70.3°.

Anal. Calcd for $C_{24}H_{38}O_6$: C, 68.24; H, 9.01. Found: C, 68.23; H, 9.03.

Dihydrohydroxyancepsenolide.—A solution of hydroxyancepsenolide (0.369 g, 0.972 mmol) in ethyl acetate (170 ml) was stirred under hydrogen at atmospheric pressure and room temperature in the presence of prereduced platinum oxide (0.178 g). A fine white precipitate was apparent in the reaction mixture by the time hydrogen uptake ceased after the absorption of slightly more than 1 mol equiv of gas. The catalyst was removed by filtration and washed with warm ethyl acetate to remove the precipitated product. Evaporation of the solvent left a white solid. The mixture of diastereomers expected in this reaction could not be resolved by fractional crystallization, nor could any separation of isomers be detected by tlc under the conditions employed. A sample recrystallized five times from ethyl acetate (29 mg from 148 mg) exhibited a melting point range of 119.3–133.0°. The material recovered from the mother liquors of the first recrystallization attempt had a melting point range of 118.8–125.5° after three recrystallizations from ethyl acetate: ir ($CHCl_3$) 3500, (OH) and 1765 cm^{-1} (saturated γ-lactones); ir (KBr) 3450 (OH), 1760, 1730 (C=O); nmr ($CDCl_3$, sparingly soluble) δ 4.10–4.80 (overlapping multiplets, 3, —CHOHCH(—O⁻), —CHOHCH(—O⁻), —CH_2CH(—O⁻)).

Anal. Calcd for $C_{22}H_{38}O_5$: C, 69.11; H, 9.95. Found: C, 68.90; H, 9.98.

Dihydrohydroxyancepsenolide Acetate.—Acetylation of dihydrohydroxyancepsenolide (0.104 g, 0.271 mmol), mp 117–133°, with pyridine–acetic anhydride as described above for II afforded a solid acetate (0.121 g) which was recrystallized once from isopropyl alcohol and then twice from carbon tetrachloride–hexane to give 57 mg of material: mp 71.2–72.5°; ir ($CHCl_3$) 1765 (saturated lactones) and 1740 cm^{-1} (acetate); nmr ($CDCl_3$) 5.12 (dd, 1, —CHOAcCH(—O⁻)—), 4.15–4.95 (overlapping complex quartets, 2, —CHOAcCH(—O⁻)— and —CH_2CH(—O⁻)—), 2.1 (s, 3, —OC(—O⁻)CH_3).

Dehydration of Hydroxyancepsenolide.—Hydroxyancepsenolide (0.253 g, 0.665 mmol) was stirred overnight with a mixture of 0.2 ml of phosphorus oxychloride and 11 ml of pyridine. The reaction mixture was diluted with four volumes of water and the product recovered by extraction into ether. The ether solution was washed with dilute hydrochloric acid and water and dried ($MgSO_4$). Evaporation of the solvent left a white solid (0.143 g, 60%) which tlc and nmr analysis indicated to be ancepsenolide. The crude product was filtered through silica gel and crystallized once from chloroform to give 0.103 g of ancepsenolide: mp 92.8–94.3°; no depression on admixture with authentic ancepsenolide; $[\alpha]^{24}D$ +7.7°. The infrared and mmr spectra of the dehydration product were identical with those of authentic ancepsenolide.[2]

Registry No.—II, 18634-45-2; acetate of II, 18634-46-3; dihydro derivative of II, 18634-47-4; acetate of dihydro derivative of II, 18634-48-5.

Acknowledgments.—We are pleased to acknowledge the use of the collecting facilities of the Institute of Marine Science, University of Miami, Miami, Fla., and the Lerner Marine Laboratory of the American Museum of Natural History, Bimini, Bahamas, W. I. We wish to thank Mr. Paul Fenessey, Massachusetts Institute of Technology, and Dr. Ronald Grigsby, Continental Oil Co., Ponca City, Okla., for providing mass spectra. This work was supported by NIH Training Grant 5675 from the National Heart Institute.

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(6) L. S. Ciereszko, D. H. Sifford, and A. J. Weinheimer, *Ann. N. Y. Acad. Sci.*, **90**, 917 (1960).

(7) The sample used to determine the physical properties was isolated from the extracts of a batch of *Pterogorgia anceps* colonies. We have previously noted[2b] variation in the optical rotation of samples of ancepsenolide isolated from different batches of dried animal. We have since observed that a sample of ancepsenolide isolated from a single animal colony of *Pterogorgia anceps* exhibited a rotation of +12.03° (+13.2° originally reported[2b]), while a sample of ancepsenolide isolated from a single colony of another species of this same genus, *Pterogorgia guadalupensis*, exhibited a rotation of +47.9°. All of the above samples appeared to be homogeneous as judged by tlc and nmr, and were found to be identical by virtue of mixture melting points as well as ir, uv and nmr spectral comparisons. Thus the correct value for the optical rotation of ancepsenolide and hydroxyancepsenolide remains uncertain. More individual colonies will be examined in the hope of clarifying this question.

Synthesis and Characterization of Cholesterol β-D-Glucuronide and Derivatives[1]

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Received November 13, 1968

The recent isolation of cholesterol sulfate from human blood plasma[2] and from the urine of normal men[3] has

(1) This work was supported by a research grant, AM 01255, from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Public Health Service.

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