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EUPACHLORIN ACETATE, A NOVEL CHLORO-SESQUITERPENOID LACTONE TUMOR INHIBITOR FROM EUPATORIUM ROTUNDIFOLIUM[1,2]

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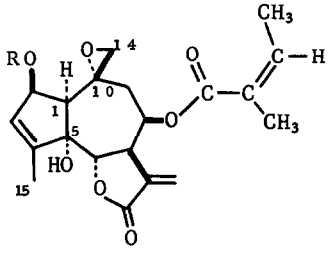
In the course of a continuing search for tumor inhibitors of plant origin, alcoholic extracts of Eupatorium rotundifolium L. (Compositae) showed significant inhibitory activity in vitro against cells derived from human carcinoma of the nasopharynx (KB).[3,4] Our earlier report described the isolation and structural elucidation of euparotin acetate (1), a novel tumor-inhibitory guaianolide sesquiterpene, and of the alcohol, euparotin (2).[3] We report herein the isolation and structural elucidation of a second tumor inhibitor, eupachlorin acetate (3), and of five additional cytotoxic[5] guaianolide lactones. Three of the lactones appear to be the first recognized naturally-occurring chloro-sesquiterpenes.

The six new lactones were isolated from the same cytotoxic fractions of the plant material which yielded euparotin acetate and euparotin. The key separations were effected by repeated silica gel chromatography with chloroform-acetone-ethanol mixtures. While all of the lactones were isolated in sufficient quantity for in vitro evaluation of cytotoxicity, only eupachlorin acetate was available in a quantity sufficient for in vivo testing. Eupachlorin acetate showed significant reproducible activity against the Walker 256 intramuscular carcinosarcoma in rats at 250-300 mg/kg.[4]

Eupachlorin acetate (3) ($C_{22}H_{27}O_8Cl$,[6] mp 161-164° (vac, dec); $[\alpha]_D^{26}$ -192° (c 0.63, MeOH); λ_{max}^{MeOH} 212 mμ (ε 15,800); λ_{max}^{KBr} 2.94, 3.40, 5.65 (lactone), 5.75 (acetate), 5.81 (angelate), 6.01 (C=C) and 8.14μ) was converted to euparotin acetate (1)[7] in 68% yield by chromatography upon acid washed alumina. Location of the chlorine atom at C-14 was supported by the downfield shift of the two-proton singlet (to τ 6.47) corresponding to the C-14 methylene of eupachlorin acetate (3), relative to the signal for the C-14 methylene (at τ 7.32) in the nmr spectrum of euparotin acetate (1). Furthermore, the tertiary nature of the two D_2O-exchangeable hydroxyl functions of 3 (and, consequently, their location at C-5 and C-10) was indicated by their resistance to acetylation with acetic anhydride-pyridine. Since there have been no apparent



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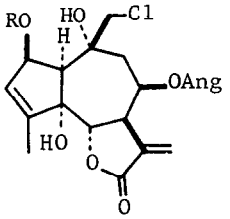
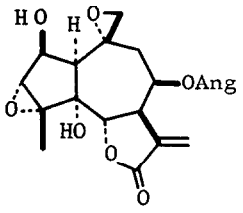
2 R = H





3 R = Ac

4 R = H

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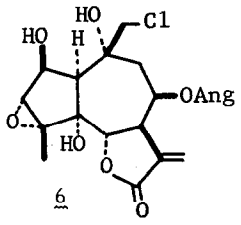
prior reports of isolation of chloro-sesquiterpene natural products, the possibility was considered that eupachlorin acetate may have been formed during the isolation procedure, by addition of the elements of hydrogen chloride to euparotin acetate. However, when a separation procedure was devised which rigorously excluded chlorine-containing salts and solvents, eupachlorin acetate was isolated in good yield. This fact and the well-documented co-occurrence in the Compositae of polyacetylenic epoxides, chlorohydrins, and chlorohydrin acetates[8] support the view that chloro-sesquiterpenes are, indeed, naturally-occurring compounds.

Eupachlorin (4) ($C_{20}H_{25}O_7Cl$, mp 219-221° dec; $[\alpha]_D^{27}$ -110° (c 0.35, EtOH); nmr signals (d_6-DMSO) at τ 6.14 and 6.37 (2H, doublets, J = 11.5 cps, C-14); 3 exchangeable hydroxyl protons) was converted to euparotin (2) by alumina chromatography and to eupachlorin acetate (3) by acetylation.

Eupatoroxin (5) ($C_{20}H_{24}O_8$, mp 197-200°; $[\alpha]_D^{26}$ -98° (c 1.10, MeOH); nmr signals (d_6-acetone) at τ 6.64 (1H, br s, C-3), 7.32 and 7.43 (2H, doublets, J=5 cps, C-14), 8.42 (3H, s, C-15)) was identical to the major product (60%) obtained by epoxidation of euparotin (2) with m-chloroperbenzoic acid. Evidence which favors the designated α-orientation of the 3,4-epoxide is discussed below.

Eupachloroxin (6) ($C_{20}H_{25}O_8Cl$) was isolated as a chromatographically-homogeneous amorphous solid. The nmr spectrum (d_6-acetone) showed signals which supported the presence of a 3,4-epoxide (τ 6.63, 1H, br s), a chlorine atom at C-14 (τ 6.19 and 6.34, 2H, doublets, J=12 cps), two tertiary hydroxyl groups (τ 5.67, 1H, s, and τ 6.03, 1H, s) and one secondary hydroxyl group (τ 5.22, d, J=4 cps). Eupachloroxin was converted to eupatoroxin (5) upon chromatography on alumina.

Eupatundin (7) ($C_{20}H_{24}O_7$, mp 188-189° (vac); $[\alpha]_D^{29}$ -80° (c 0.44, EtOH))



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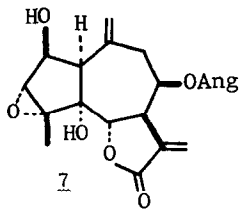

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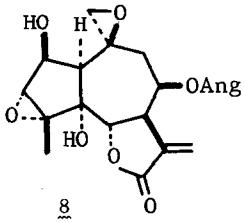

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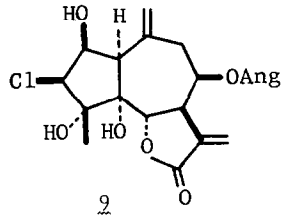

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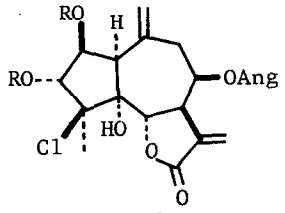

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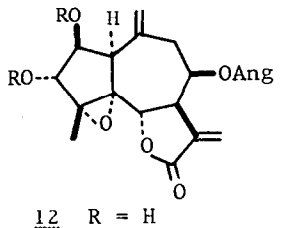
11 R = Ac



12 R = H
13 R = Ac

showed nmr signals which indicated the presence of a 3,4-epoxide (τ 6.58, 1H, br s, and 8.35, 3H, s, C-15) and an exocyclic methylene group at C-10 (τ 4.94, 2H, s). Treatment with m-chloroperbenzoic acid gave a mixture of epoxides from which eupatoroxin (5) was isolated in 8% yield. A second reaction product (25%) was identical to the sixth naturally-occurring lactone, characterized as 10-epi-eupatoroxin (8) ($C_{20}H_{24}O_8$, mp 230-232°; $[\alpha]_D^{26}$ -109° (c 0.33, MeOH); nmr signals (d_6-acetone) at τ 6.62 (1H, br s, C-3), 7.24 and 7.33 (2H, doublets, J=5 cps), two exchangeable hydroxyl protons).

Assignment of α-configuration to the 3,4-epoxide in eupatundin (7) is favored by the experimental results which follow. Treatment of 7 in aqueous dioxane with hydrogen chloride gas gave two chlorohydrins, 9 (72% yield, $C_{20}H_{25}O_7Cl$, mp 260-262° dec, m/e 412, 414 (3:1, M^+); $[\alpha]_D^{27}$ +50° (c 0.10, MeOH); nmr signals (d_5-pyridine) at τ 5.22 (1H, d, J=3.5 cps, C-3), 5.72 (1H, m), 7.80 (3H, s, C-15), and three exchangeable hydroxyl protons) and 10 (20% yield, $C_{20}H_{25}O_7Cl$, mp 190-192° dec, m/e 412, 414 (3:1, M^+); $[\alpha]_D^{27}$ -50° (c 0.86, MeOH); and nmr signals (d_6-acetone) at τ 5.39 (1H, d, J=8 cps, C-3), 5.69 (1H, t, J=8 cps, C-2), 8.30 (3H, s, C-15), and three exchangeable hydroxyl protons). Acetylation of 10 gave diacetate 11 ($C_{24}H_{29}O_9Cl$, m/e 496, 498 (3:1, M^+); nmr signals (d_6-acetone) at τ 3.94 (1H, d, J=7 cps, C-3), 4.76 (1H, t, J=7 cps,

C-2), 7.82, 7.98 (6H, two $COCH_3$), 8.28 (3H, s, C-15), and one exchangeable hydroxyl proton). Alumina treatment of 9 gave only eupatundin (7), whereas similar treatment of 10 gave 7 (6.5%) and a new epoxide, 12 (90%)($C_{20}H_{24}O_7$, m/e 376 (M^+); nmr signals ($CDCl_3$) at τ 5.88 (2H, m, C-2, C-3), 8.42 (3H, s, C-15) and two exchangeable hydroxyl protons). Acetylation of 12 gave diacetate 13 ($C_{24}H_{28}O_9$, m/e 460 (M^+); nmr signals ($CDCl_3$) at τ 4.25 (1H, m, C-3), 5.21 (1H, t, J=7.5 cps, C-2), 7.79 and 8.00 (6H, two $COCH_3$), 8.44 (3H, s, C-15), and no exchangeable hydroxyl protons). A 3,4β-epoxide would have been expected to yield 3α- and 4α-chloro chlorohydrins, and the 3α-chloro compound would have been expected to close, in part, to the unnatural (and un-acylable) 2,3β-epoxide. The foregoing experimental results strongly support the view that the 3,4-epoxide is α-oriented in eupatundin (7) and related compounds.

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2. Supported by grants from the National Cancer Institute (CA-04500), the American Cancer Society (T-275), and a contract with the Cancer Chemotherapy National Service Center (C.C.N.S.C.), National Cancer Institute, National Institutes of Health (PH-43-64-551). JEK was N.I.H. Predoctoral Fellow, 1965-68. JMC was N.I.H. Postdoctoral Fellow, 1965-66.
3. S.M. Kupchan, J.C. Hemingway, J.M. Cassady, J.R. Knox, A.T. McPhail, and G.A. Sim, J. Am. Chem. Soc. 89, 465 (1967).
4. Cytotoxicity against KB (human carcinoma of the nasopharynx) cell culture and in vivo inhibitory activity against Walker 256 intramuscular carcinosarcoma in rats were assayed under the auspices of the C.C.N.S.C., by the procedures described in Cancer Chemotherapy Rept. 25, 1 (1962).
5. The isolated lactones showed cytotoxicity (ED_{50}) against KB cell culture at the indicated concentrations: eupachlorin acetate (3), 0.18 μg/ml; eupachlorin (4), 0.21 μg/ml; eupatoroxin (5), 2.8 μg/ml; eupachloroxin (6), 3.6 μg/ml; eupatundin (7), 0.39 μg/ml; 10-epi-eupatoroxin (8), 2.6 μg/ml. A compound is considered active in the C.C.N.S.C. assay if the average $ED_{50} \leq 4$ μg/ml.
6. Cited empirical formulas were supported by satisfactory analyses and/or mass spectral molecular weights. We thank Dr. G. Van Lear and Dr. F. W. McLafferty of the Purdue Mass Spectrometry Center, supported under U.S. Public Health Service grant FR-00354, for the mass spectral data.
7. The identity of materials was established by mp, mixture mp, tlc, mixture tlc, optical rotation, high resolution ir, and nmr spectral comparisons.
8. F. Bohlmann, et al., Chem. Ber. 95, 2945 (1962); 97, 520, 809 (1964).