ANTINEOPLASTIC AGENTS. 81. THE GLYCEROL ETHERS OF *PALYTHOA LISCIA*[1]

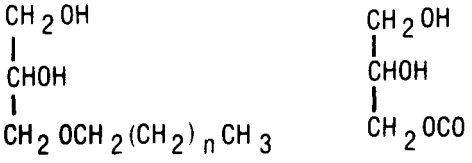
GEORGE R. PETTIT* and YOUICHI FUJII

Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85287

Brain astrocytoma- glioblastoma- and oligodendroglioma-type neoplasma have been found to produce neutral lipid fractions with abnormally high (45% increase over normal brain tissue) amounts of alkyl (1.4–4.8% of the total) and alkenyl (3.8–10.1%) diacyl glycerols (2). Similar conclusions have been reached with a cross-section of human and animal cancers and neoplastic cell lines (3–9). Conversely, the naturally occurring[2] 1-*O*-hexadecyl-glycerol (**1a**, chimyl alcohol) and 1-*O*-octadecyl-glycerol (**1b**, batyl alcohol) have been shown to slightly inhibit progress of the murine Ehrlich ascites tumor. The synthetic 1-*O*-decyl- and 1-*O*-dodecyl-glycerols caused marked inhibition of the Ehrlich tumor (16).[3]

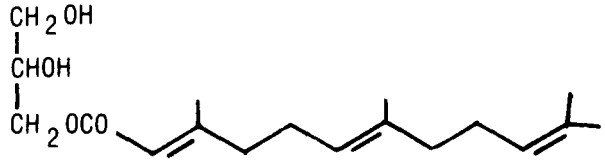
Interestingly, batyl alcohol (**1b**) has been found to accelerate wound healing (17), be protective against radiation sickness (16), and exhibit antiinflammatory activity similar (in the rat) to hydrocortisone (20). A glycerol ether fraction from cod liver oil has shown antibacterial activity (21) and farnesic acid ester **2** from the nudibranch *Archidoris odhneri* has displayed reasonable antibiotic activity against *Staphylococcus aureus* (22).

An investigation aimed at isolating the cell growth (murine P388 lymphocytic leukemia, PS system, 23) inhibitory constituents of the Western Indian Ocean (Mauritius) *Palythoa liscia* Haddon and Duerden led to discovery of the PS active (*in vitro*) palystatins 1–3 (24) and palystatins A–D (25). As part of these efforts the glycerol ether components of a ligroin fraction (PS ED_{50} 0.48 μg/ml) were also explored.[4] For this purpose the ethanol extract of a 1975 recollection of *Palythoa liscia* Haddon and Duerden was partitioned between chloroform-water, and the chloroform



1 a, n = 14 (chimyl alcohol)

b, n = 16 (batyl alcohol)



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[1]For part 80 refer to reference (1).

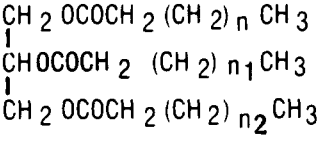
[2]In 1921 Tsujimoto and Toyama reported (10,11) that batyl and selachyl (1-*O*-octadecenyl-glycerol) alcohols were a common component of an unsaponifiable fraction of certain elasmobranch (sharks and rays) liver oils and the structures were ascertained in the period 1930–33 (12–14). About 10 years later the absolute configurations of both batyl and chimyl alcohols were determined by synthesis from (−) glycerol acetonide (15). Glycerol ethers have been located in other such diverse sources as human fetal meconium, milk, bone marrow, spleen and arteriosclerotic tissue (for leading references consult 16), soft coral (**1b**, 17), various marine mollusca (**1a**,**b**, 18), and the preen gland of a plumed egret (**1a**,**b**, 19).

[3]Some detergents interfere with cell membranes and lead to cell lysis. Since glyceryl ethers display weak surface activity this might explain, in part, the Ehrlich ascites activity (16).

[4]The sterols of *Palythoa mammilosa* and *P. tuberculosa* have been isolated (26). A series of unique dehydroamino acids were found (27,28,29) to be produced by *P. tuberculosa*, and the yellow zoanthoxanthins and pseudozoanthoxanthins of five *Palythoa* species have been determined (30).

fraction was partitioned between methanol-water (9:1) and ligroin (24).

The preceding ligroin residue (127 g) was chromatographed on silica gel. A ligroin-acetone (3:7) fraction was rechromatographed on silica gel. The fraction eluted by benzene-ethyl acetate (1:4) was further purified by partition chromatography on a column of Sephadex LH-20 [ligroin-methylene chloride-ethanol (10:10:1)]. Recrystallization of fractions from the LH-20 separation afforded chimyl alcohol (**1a**, 48 mg) and batyl alcohol (**1b**, 92 mg) as the only significant glycerol ether components. Another fraction from a silica gel chromatographic separation (eluted by ligroin-acetone) was chromatographed on silica gel, and a benzene fraction reprecipitated from acetone provided (50 mg) of a product that displayed only one spot upon thin-layer chromatography. But a field desorption mass spectral study indicated that this fraction was a mixture of glyceryl tripalmitate (**3a**), a glyceryl palmitate distearate (**3b** or **c**), and a glyceryl stearate dipalmitate (**3d** or **e**).



3 a, $n = n_1 = n_2 = 13$

b, $n = 13$, $n_1 = n_2 = 15$

c, $n = n_2 = 15$, $n_1 = 13$

d, $n = n_1 = 13$, $n_2 = 15$

e, $n = n_2 = 13$, $n_1 = 15$

When the glyceryl ethers (**1a** and **b** and a number of related compounds prepared by synthesis),[5] the triglycerides (**3**) and other lipid-type fractions were found to be inactive against both the *in vitro* and *in vivo* PS system this avenue of the *Palythoa* problem was discontinued. Perhaps, as noted above for the Ehrlich carcinoma, such glycerol ethers may prove useful against certain experimental tumor systems other than the PS. Meanwhile, alkyl phospholipids of this type may find an important role in monitoring malignant advance and regression.

EXPERIMENTAL

ANIMAL COLLECTION, EXTRACTION AND PRELIMINARY SEPARATION.—A 25 kg (approximate wet weight) amount of a *Palythoa liscia* Haddon and Duerden (Coelenterata phylum, Zoanthidae family) was recollected (first collected in 1972) in 1975 at Tombeau Bay, Mauritius, and extracted with ethanol (24,25).[6] The ethanol extract (835 g) was partitioned between chloroform-water and the chloroform (24) residue (190 g) was further partitioned with methanol-water (9:1)-ligroin. Evaporation of the ligroin yielded 127 g of brown oil.

CHROMATOGRAPHY.—Each of the chromatography solvents was redistilled. Column chromatography was performed with silica gel (70–230 mesh) from E. Merck, Darmstadt, West Germany, and with Sephadex LH-20 from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Thin-layer chromatography was performed with silica gel GF Uniplates supplied by Analtech, Inc. The thin-layer plates were visualized by spraying with concentrated sulfuric acid and heating or by exposure to iodine vapor.

INSTRUMENTAL ANALYSIS.—All melting points are uncorrected and were observed on a Kofler-type melting point apparatus. The optical rotations were kindly provided by Dr. C. Herald employing a Perkin-Elmer model 241 spectrophotometer. The infrared and ^{1}H nmr spectra (4% solution in deuteriochloroform with tetramethylsilane as internal standard) were provided by Dr. J. Witschel, Jr., using a Beckman model 12 (infrared) and Varian XL-100 (NMR) instruments. Mass spectra were obtained by Dr. P. Brown and Mr. E. Kelley employing MAT CH-4B and 112S (equipped for electron impact or field desorption) instruments.

The authentic specimens of chimyl alcohol (**1a**) and batyl alcohol (**1b**, from dog fish liver oil) employed for comparison purposes were obtained from Supelco, Inc., Bellefonte, Pa., and Sigma Chemical Co., respectively.

ISOLATION OF (+)-1-O-*n*-HEXADECYL-GLYCEROL (**1a**) AND (+)-1-O-*n*-OCTADECYL-GLY-

[5]Unpublished experiments.

[6]A specimen of this animal has been deposited with the Smithsonian Institution.

CEROL (**1b**).—The 127 g of ligroin partitioning product (see above), chromatographed on silica gel (1.04 kg) and eluted successively with ligroin (2 liters), and ligroin-acetone (9:1, 1.5 liters), ligroin-acetone (7:3, 2 liters), ligroin-acetone (3:7, 2 liters), gave in that order fractions 1–4. Fraction 3 (37 g) was rechromatographed on silica gel (1 kg) with a gradient of benzene-ethyl acetate. The fraction (9.3 g) eluted by benzene-ethyl acetate (1:4), when further chromatographed on Sephadex LH-20 (113 x 4 cm) with ligroin-methylene chloride-ethanol (10:10:1), gave five principal fractions. The second (0.75 g) was reprecipitated (3x) from methanol, once from ligroin and once from acetone to give batyl alcohol (**1b**, 92 mg) as a colorless amorphous solid: mp 66–69°; $[\alpha]^{25}D$+1.47° (c, 2.04 in chloroform). The authentic sample melted at mp 67–69°; (Ref. 15, mp 71–77°, $[\alpha]^{20}D$+0.8°, c, 8.4 in chloroform): ir ν max (KBr) 3420, 3350, 2930, 2860, 1460, 1130, 1090, 1055, 920, 870, 730 cm^{-1}; EI mass spectrum (70 eV) *m/e*, 313 (M–31, + $CH(OH)CH_2OCH_2(CH_2)_{16}CH_3$), 283 (M–61, + $CH_2OCH_2(CH_2)_6CH_3$), 253 (M–91, + $CH_2(CH_2)_{16}CH_3$), 225 (M–119, + $(CH_2(CH_2)_{14}CH_3$); ^1H nmr δ, 0.89 (3H, m, $-CH_3$), 1.28 (30H, broad s), 1.60 (2H, m), 2.28 (1H, m, –OH), 2.68 (1H, m, –OH), 3.58 (6H, m, $HOCH_2CH(OH)CH_2OCH_2-$), 3.86 (1H, m, $HOCH_2CH(OH)CH_2O-$).

Recrystallization (4x) of the fourth LH-20 partition fraction from methanol afforded chimyl alcohol (**1a**, 48 mg) as colorless needles: mp 65–67°; $[\alpha]^{20}D$+1.22° (c, 0.82 in chloroform). The authentic sample melted at 66–67°; (Ref. 15, mp 62.5–63.5°, $[\alpha]^{20}D$+3.0°, c, 1.16 in chloroform): ir ν max ($CHCl_3$) 3440, 2940, 2860, 1465, 1225, 1120 cm^{-1}; EI mass spectrum (70 eV) *m/e* 285 (M–31, + $CH(OH)CH_2OCH_2(CH_2)_{14}CH_3$), 255 (M–61, + $CH_2OCH_2(CH_2)_{14}CH_3$), 225 (M–91, + $CH_2(CH_2)_{14}CH_3$); ^1H nmr δ 0.87 (3H, m, $-CH_3$), 1.26 (26H, broad s), 1.60 (2H, m), 2.23 (1H, m, –OH), 2.65 (1H, m, –OH), 3.60 (6H, m, $HOCH_2CH(OH)CH_2OCH_2-$), 3.85 (1H, m, $HOCH_2CH(OH)CH_2O-$).

Mixture melting point determinations of alcohols **1a** and **b** with the respective authentic samples showed no depression. The ir (KBr), ^1H nmr and mass spectra as well as the thin layer chromatographic behavior of the *Palythoa* chimyl (**1a**) and batyl (**1b**) alcohols was identical with the analogous authentic sample data.

SEPARATION OF A TRIGLYCERIDE FRACTION (**3**).—The second fraction (30 g) from the original silica gel chromatogram (see above) was rechromatographed on silica gel (430 g) using a benzene-ethyl acetate gradient. A fraction (0.31 g) eluted by benzene was reprecipitated (4x) from acetone to give the triglycerides (**3**, 50 mg) as a colorless amorphous solid melting at 56–61°. Thin-layer chromatography of this mixture with e.g., benzene as mobile phase[7] gave a single spot. However, an FD mass spectrum showed three components, *m/e* 862 (M^+, **3b** or **c**), 834 (M^+, **3d** or **e**), 806 (M^+, **3a**). The position of the fatty acids in esters **3b-e** was not determined, but **3a** was characterized as glyceryl tripalmitate. EI mass and ^1H nmr spectra of the triglyceride mixture follows: mass spectrum (70 eV) *m/e*, 267 ($^{\oplus}O{\equiv}CCH_2(CH_2)_{15}CH_3$), 239 ($^{\oplus}O{\equiv}CCH_2(CH_2)_{13}CH_3$); ^1H nmr (3% solution in $CDCl_3$) δ 0.88 (9H, m, $-CH_3$), 1.27 (broad s, $-CH_2-$), 1.60 (6H, m, $-COCH_2-CH_2-$), 2.33 (6H, t, J=7 Hz, $-COCH_2CH_2-$), 4.24 (4H, m, $-COOCH_2-CH-CH_2OCO-$), 5.28 (1H, m, $-COOCH_2-CH-CH_2OCO-$).

[7]Such mixtures may be separated, in part, by complexing with silver ion on a thin layer plate (31).

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