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3-D-Hydroxypalmitic acid: a metabolic product of the yeast NRRL Y-6954

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An extracellular lipid produced by the yeast NRRL Y-6954 was shown to be free 3-D-hydroxypalmitic acid. The structure of the hydroxy acid was established by the mass spectrum of its methyl ester and a direct comparison with an authentic sample of methyl 3-D-hydroxypalmitate.

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The formation of extracellular lipids of yeasts has recently been reviewed by Stodola *et al.* (1).

In continuing work in this area, we found that free 3-D-hydroxypalmitic acid (**1**) is excreted by an unidentified yeast. In 1964, Tulloch and Spencer (2), and van Ammers *et al.* (3) reported that this acid was a component of glycolipids produced by *Rhodotorula* species.

Acid **1** was produced in a yield of 1.76 g/l by growing the yeast for 4.5 days at 25 °C in shaken flasks on a medium containing 5% malt extract and 2% Cerelose.[1] The crude acid (m.p. 70–80 °C), obtained by extraction of the culture liquor with ether, was shown by thin-layer chromatography to consist largely of one component.

Crystallization from hexane gave pure acid **1** (m.p. 79.5–80.5 °C) of formula $C_{16}H_{32}O_3$, the infrared (i.r.) spectrum of which showed hydroxyl absorption (3565 cm^{-1} (w)) and carbonyl absorption (1710 cm^{-1} (s)) in 1% chloroform. Methyl (**2**) and *p*-bromophenacyl (**3**) esters gave analytical values in accord with the $C_{16}H_{32}O_3$ formula.

The mass spectrum of methyl ester **2** was directly comparable with the mass spectrum of methyl 3-DL-hydroxyoctadecanoate observed by Ryhage and Stenhagen (4). The position of the hydroxyl group on the carbon chain is indicated by a peak at mass 103; the highest in the spectrum, this peak is due to the fragment CH_3OCCH_2CHOH obtained from cleavage of the 3,4 carbon–carbon bond. The following comparisons were also found: peaks at 194, 236 [M − 32 − 18(CH_3OH,H_2O)], and 268 [M − 18(H_2O)] for methyl ester **2**, are 28 mass numbers less than peaks 222, 264 [M − 32 − 18(CH_3OH,H_2O)], and 296 [M − 18(H_2O)] found by Ryhage and Stenhagen for methyl 3-DL-hydroxyoctadecanoate.

The mass spectrum of **2** and its direct comparison (i.r. and m.p.) with methyl 3-D-hydroxypalmitate, kindly supplied by Dr. A. P. Tulloch, established the structure of **1**.

Experimental

Melting points were determined with a Fisher–Johns apparatus and are not corrected. The infrared spectra were recorded on a Perkin–Elmer (model 137), and a Beckman (model IR8) infrared spectrophotometer. The mass spectrum was recorded on a Nuclide instrument (model 12-90 G).

Production of Crude Acid (1)

The stock culture of the yeast (NRRL Y-6954 in the ARS Culture Collection here) was maintained at 25 °C on YM agar slants (1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, and 2% agar). A sterilized liquid medium (200 ml), consisting of 2% Cerelose and 5% malt extract contained in 500-ml Erlenmeyer flasks, was inoculated by loop with cells from 1-day-old YM slant cultures. The flasks were kept on a Gump shaker at 25 °C.

At intervals the contents of five flasks were extracted with ether. The yields of crude acid were: 2.5 days, 212

[1]Mention of firm names or trade products does not constitute endorsement by the United States Department of Agriculture over other firms or products not mentioned.

mg; 3.5 days, 326 mg; 4.5 days, 353 mg; 5.5 days, 331 mg; 6.5 days, 312 mg. The maximum at 4.5 days corresponded to a yield of 1.76 g/l.

3-D-Hydroxypalmitic Acid (1)

Crude acid (4.00 g, m.p. 70–80 °C) was crystallized from hexane (3.55 g, m.p. 78.8–79.8 °C). A small amount of yellow impurity (1%) was removed by charcoal. A second crystallization (200 mg) gave pure acid **1** (180 mg, m.p. 79.5–80.5 °C). $[\alpha]_D^{24}$ −13.6° (*c*, 1.3, $CHCl_3$); lit. (2), m.p. 78–79 °C, $[\alpha]_D^{25}$ −12.9° (*c*, 1.3, $CHCl_3$).

Anal. Calcd. for $C_{16}H_{32}O_3$: C, 70.54; H, 11.84. Found: C, 70.5; H, 11.9.

Methyl 3-D-Hydroxypalmitate (2)

Acid **1** (200 mg) was methylated with diazomethane. Crude ester (210 mg, m.p. 46.5–49 °C) was crystallized from hexane (160 mg, m.p. 49–49.8 °C) in fine needles. Infrared spectra of films deposited on KRS-5 plates showed hydroxyl absorption at 3390 cm^{-1} and 3300 cm^{-1} and carbonyl absorption at 1740 cm^{-1} and 1695 cm^{-1}.

Anal. Calcd. for $C_{17}H_{34}O_3$: C, 71.28; H, 11.96. Found: C, 70.9; H, 12.0.

p-Bromophenacyl 3-D-Hydroxypalmitate (3)

Acid **1** (100 mg) was converted to the *p*-bromophenacyl derivative in acetone (15 min at 70 °C) according to the DICE procedure (5). The crude product (167 mg, m.p. 108–109 °C) on crystallization from 95% ethanol gave 152 mg of *p*-bromophenacyl ester (111–111.5 °C).

Anal. Calcd. for $C_{24}H_{37}O_4Br$: C, 61.40; H, 7.94. Found: C, 61.5; H, 8.0.

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Imidazo(1,2-*a*)indole and *s*-triazolo(2,3-*a*)indole derivatives by intramolecular cyclization of 1-(*o*-acetylphenyl)azoles[1]

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9-Hydroxy-9-methyl derivatives of the ring systems named in the title are formed along with the expected 1-(*o*-acetylphenyl)azoles in the Ullmann condensations of *o*-bromoacetophenone with imidazole and with 1,2,4-triazole. The structures of these compounds follow from their characteristic proton magnetic resonance spectra.

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Common electrophilic substitution reactions with imidazole give 4(5)-substitution (1), although diazo coupling in alkaline media occurs at the 2-position (2). Reports of initial iodination at the 2-position are incorrect (3). These observations may be rationalized by assuming that 4(5)-substitution involves reaction of the conjugate acid, while 2-substitution involves the neutral molecule or the anion of imidazole (4). *N*-vinylimidazole undergoes 2-hydroxymethylation when heated with formaldehyde (5), and other *N*-substituted imidazoles undergo condensation with aldehydes to yield 2-(*N*-imidazolyl)-carbinols, while 4-alkylimidazoles give 4-alkyl-5-hydroxymethyl derivatives with formaldehyde (6).

In contrast, there is but one report classifiable as an electrophilic substitution in 1,2,4-triazoles; 4-phenyl-1,2,4-triazole is reported to undergo benzoylation in the 3-position by heating with benzoyl chloride (7). 1,2,3-Triazoles have been brominated (8) and nitrated (9) successfully.

This note reports some examples of intramolecular electrophilic substitution in imidazole and 1,2,4-triazole derivatives, leading to derivatives, of the imidazo(1,2-*a*)indole and *s*-triazolo-(2,3-*a*)indole ring systems.

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