2-D-Hydroxyhexadecanoic acid: a metabolic product of the yeast *Hansenula sydowiorum*

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A new species of yeast, *Hansenula sydowiorum*, excreted free α-hydroxy fatty acids when grown on glucose solution. The major component, 2-D-hydroxyhexadecanoic acid, was identified by the mass spectrum of its methyl ester.

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The occurrence of α-hydroxy fatty acids in plants, animals, yeasts, molds, and bacteria was reviewed by Downing (1) in 1961. Since that time additional reports have appeared on the isolation of these acids from the microorganisms *Claviceps purpurea* (2), *Rhodomicrobium vannielii* (3), a strain of *Streptomyces* (4), and *Streptomyces sioyaensis* (5). These higher α-hydroxy acids always occur as constituents of more complex molecules, usually in the form of esters and amides (6).

We recently observed that free α-hydroxy acids are formed by a new species of yeast, *Hansenula sydowiorum*, isolated (7) in South Africa from the insect *Sinoxylon ruficorne* Fahr., which we examined during our work on the extracellular lipids of yeasts (8). When grown for 12 days on a malt-glucose medium, the bisexual form of this organism produced 1.66 g/l of crude acid, 80% of which was 2-D-hydroxyhexadecanoic acid. The unisexual form secreted only one-half this amount of acid into the culture medium.

The crude acid mixture, obtained by ether extraction of the culture liquor, was converted to methyl esters with diazomethane. Preparative gas–liquid chromatography (g.l.c.) yielded as the main component methyl 2-hydroxyhexadecanoate (m.p. 45–45.5 °C, lit. (9), 45.5–45.7 °C); its levorotation in chloroform (−5.8°; *c*, 6.4) shows the free acid to have the D-configuration, as do all known naturally occurring 2-hydroxy acids. Our value for the rotation of this ester is intermediate between that of Hitchcock *et al.* (10) (−8.0°; *c*, 2.5, $CHCl_3$) and that of Horn *et al.* (9) (−3.6°; *c*, 10, $CHCl_3$), showing the dependence of the value on concentration. Baer and Mahadevan (11) reported a similar effect for 1,2-diglycerides in chlorinated solvents.

The structure of the methyl ester was established by showing the identity of its mass spectrum with that of methyl 2-DL-hydroxyhexadecanoate. Saponification of the pure ester gave 2-D-hydroxyhexadecanoic acid; m.p. 91–92 °C, lit. (9), 93.3–93.5 °C; $[\alpha]_D^{24}$ +4.6 (*c*, 6.1, pyridine), lit. (9), +3.6° (*c*, 6, pyridine). The *p*-bromophenacyl ester melted at 87.5–88 °C.

Experimental

Melting points were determined with a Fisher–Johns[2] apparatus and were not corrected. Mass spectra were recorded on a Nuclide instrument (model 12-90 G). Gas chromatographic analyses were carried out in an F and M laboratory chromatograph (model 700) equipped with a flame ionization detector. The column (4 ft × 1/4 in. o.d. aluminum) was packed with 20% silicone OV-17 on Chromosorb W-HMDS. Temperature was maintained at 300 °C and helium flow was 50 ml/min. Preparative g.l.c. was carried out in an Aerograph Autoprep (model A-700) equipped with a thermoconductivity detector. The column (6 ft × 1/4 in. stainless steel) was packed with 5% Apiezon L on Chromosorb W-AWDMCS. The temperature was maintained at 200 °C.

Production of Crude Acid

Stock cultures of the yeasts [NRRL Y-7128 (unisexual) and NRRL Y-7130 (bisexual) from the ARS Culture Collection here] were 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 5% commercial glucose hydrate and 5% malt extract contained in 500 ml Erlenmeyer flasks, was inoculated with 0.1 ml of a cell suspension prepared from a 1 day old YM slant culture and 2 ml of YM broth. The flasks were incubated at 25 °C on a Gump shaker (180 r.p.m.; 1 in. radius).

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[2]The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

TABLE 1

Lipid formation by *Hansenula sydowiorum*

Days	Yield of crude lipid in mg/l	
	Y-7128 Unisexual	Y-7130 Bisexual
3	550	550
5	675	1000
8	840	1345
12	—	1665
14	575	1300

At intervals the contents of 24 flasks were extracted separately with ether. Yields of crude acid for the unisexual and bisexual yeasts are recorded in Table 1.

Methyl 2-D-Hydroxyhexadecanoate

A composite of crude acid (2.00 g) was esterified with diazomethane. The resulting ester was shown by mass spectral and g.l.c. analyses to consist of 80% methyl 2-hydroxyhexadecanoate, the remainder being C_{15}, C_{17}, and C_{18} homologs. A portion of the crude ester (110 mg, m.p. 41–42.5 °C), subjected to preparative g.l.c., yielded 62.0 mg (m.p. 44.5–45 °C) of the main component, which was crystallized from hexane (m.p. 45–45.5 °C), $[\alpha]_D^{24}$ −5.8° (*c*, 6.4, $CHCl_3$); lit. (9), m.p. 45.5–45.7 °C, $[\alpha]_D$ −3.6° (*c*, 10, $CHCl_3$).

Anal. Calcd. for $C_{17}H_{34}O_3$: C, 71.28; H, 11.96. Found: C, 71.7; H, 12.1.

p-Bromophenacyl 2-D-Hydroxyhexadecanoate

Methyl ester (55 mg), refluxed for 1 h with alcoholic KOH, gave 50 mg of crude hydroxy acid (m.p. 89–92 °C). Crystallization from hexane yielded 23 mg of acid, which was converted to the *p*-bromophenacyl derivative by the DICE procedure (12). The crude product (38 mg, m.p. 77–83 °C) on crystallization from 95% alcohol gave 22 mg of pure *p*-bromophenacyl ester (m.p. 87.5–88 °C).

Anal. Calcd. for $C_{24}H_{37}O_4Br$: C, 61.40; H, 7.94. Found: C, 61.6; H, 8.1.

A second portion of crude ester (500 mg) gave a larger sample of pure 2-D-hydroxyhexadecanoic acid, which melted at 91–92 °C; $[\alpha]_D^{24}$ of +4.6° (*c*, 6.1, pyridine).

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