11-Hydroxy-*trans*-8-dodecenoic Acid Lactone, a 12-Membered-Ring Compound from a Fungus

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The 12-membered lactone of 11-hydroxy-*trans*-8-dodecenoic acid is formed when the fungus *Cephalosporium recifei* NRRL 5161 is grown on glucose solution.

On a observé que la lactone à douze chaînons de l'acide hydroxy-11 dodécèn-8-*trans*-oïque se forme lorsque l'on fait croître les champignons du *Cephalosporium recifei* NRRL 5161 dans des solutions de glucose.

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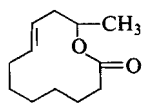
Naturally occurring large-ring lactones were first described by Kerschbaum (1) in 1927. Since that time many more examples of this class of compounds have appeared, most of them macrolides. We now report the isolation of the monocyclic lactone **1** from a fungus.



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The organism appears to belong to the group *Cephalosporium recifei*, based on morphological studies and production of yellow pigment. The fungus was grown for 35 days on a shaken malt–glucose medium. Ether extraction of the whole culture liquor gave an average of 1.7 g of a viscous yellow oil *A* per liter of culture liquor. Hexane extraction of *A* at room temperature gave a 14% yield of nonpolar oil *B*, silicic acid column purification of which yielded a colorless mobile liquid *C* (77%), shown by g.l.c. to be 95% **1**. Pure lactone **1** was obtained from fraction *C* in about 50% yield by preparative g.l.c.

The structure of **1** was established as follows: elementary analyses and the molecular ion peak of *m/e* 196 corresponded to a formula of $C_{12}H_{20}O_2$. The i.r. spectrum showed a band at 1735 cm^{-1}, indicative of either a lactone or an ester, and a band at 975 cm^{-1}, characteristic of a *trans* double bond. That the double bond was unconjugated was evident from the u.v. spectrum (end absorption only). Chemical studies are summarized in Scheme 1.

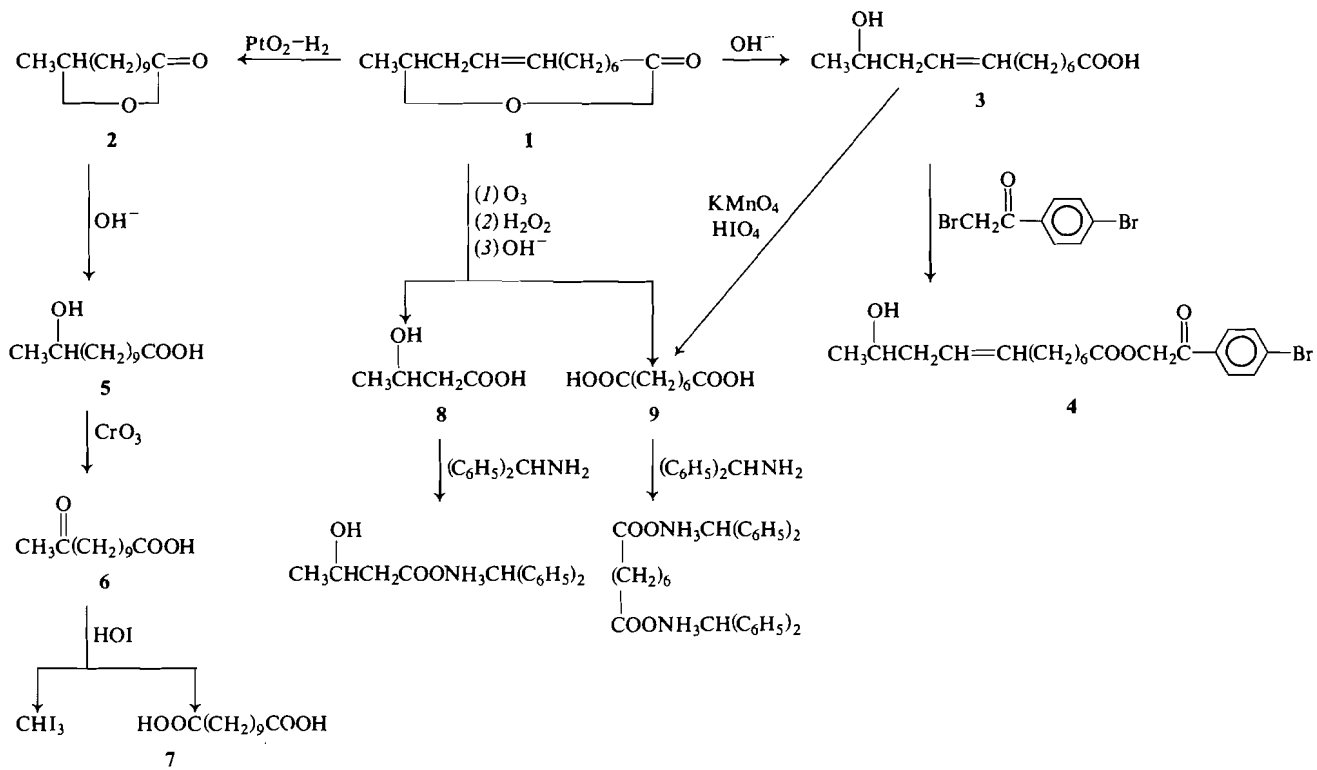
It was first established that **1** is a lactone, rather than an ester, by saponification to **3**, which was shown to be a 12-carbon acid by elementary analysis of its phenacyl derivative **4**.

Next, the size of the lactone ring was determined. The optical activity of **1** ($[\alpha]_D^{25}$ + 73.2°) required substitution on the lactone ring. The nature and the position of the substituent were arrived at by CrO_3 oxidation of **5** to the keto acid **6**, which gave iodoform and the acid **7** on treatment with hypoiodite.

Finally, the position of the double bond in **1** was ascertained by oxidation procedures (ozonolysis and $KMnO_4$–HIO_4), which led to 3-hydroxy-butyric acid (**8**) and suberic acid (**9**), both characterized as their benzhydrylamine salts.

Information on the mass spectra of simple large-ring lactones appears to be meagre, the only two compounds studied so far being cyclopentadecanolide and 12-stearolactone (2). In our work the fragmentation patterns appear to be compatible with the assigned structures. The low resolution spectra of **1** (Fig. 1) and its dihydro derivative **2** (Fig. 2) are plotted along with the atomic compositions (determined by high resolution peak matching). Large peaks occur in the spectrum of **1** at 152 and 98, due probably to rearrangements involving opening of the lactone ring and cleavage at the C_{10}—C_{11} bond to form the 152 peak and cleavage at the C_6—C_7 bond to give the 98 peak.

The 152 peak of **1** also shifts two mass units higher to 154 in **2**, while the 98 peak of **1** at the carbonyl end of the molecule remains at 98 in **2**.



SCHEME 1

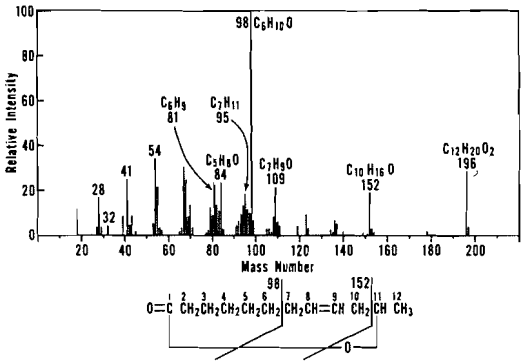


FIG. 1. Mass spectrum of lactone of 11-hydroxy-*trans*-8-dodecenoic acid (**1**).

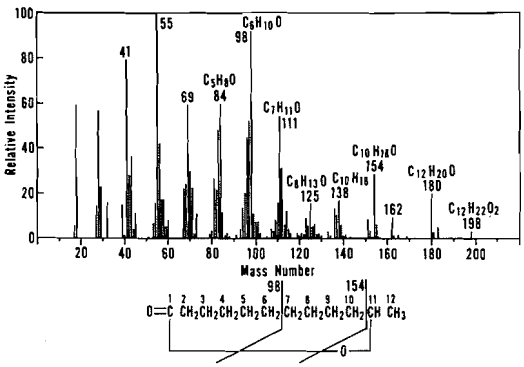


FIG. 2. Mass spectrum of lactone of 11-hydroxydodecanoic acid (**2**).

The 98 peak is the most intense peak in **1**, being three times the next highest peak; it is due to the weakening of the C_6—C_7 bond beta to the C_8—C_9 double bond. When the double bond is reduced, this cleavage is less selective and peaks appear at 84, 98, and 112 in **2**.

The mass spectrum, not shown, of the methyl ester of **3** had peaks at mass 74, 88%, and 87, 52%, characteristic of methyl esters. The molecular weight of the methyl ester is 228, but its spectrum did not show a molecular ion peak. Peaks were found at 210, 9%, due to loss of water; 184, 20%, due to loss of C_2H_4O; and at 152, 62%, due to further loss of methanol from the 184 peak. The loss of water, cleavage at the carbon-to-carbon bond adjacent to the OH, and the further loss of methanol from the chain cleavage fragment are all well-characterized fragmentations of methyl hydroxy esters, and these particular mass numbers indicate that the OH is on C-11 (3).

The simple large-ring lactones have found commercial application as fixatives in perfumes; this usage has stimulated considerable interest in their synthesis and properties (4).

Experimental

Melting points were determined on a Fisher–Johns[1] apparatus and are uncorrected. The i.r. spectra were recorded on Perkin–Elmer (Model 137) and Beckman (Model IR8) spectrophotometers. The g.l.c. analyses were carried out in a Packard 7401 gas chromatograph equipped with dual columns and independent flame-ionization detectors. Two columns were used: (*1*) 12 ft × 1/4 in.; 5% LAC-2-R 446 on Chromosorb W AW-DMCS; (*2*) 4 ft × 1/4 in., 5% Apiezon L on Chromosorb W AW-DMCS. The temperature of the inlet was 200 °C and that of the column oven was 190 °C. An Aerograph (Model 1520) gas chromatograph, equipped with a thermoconductivity detector, was used for preparative g.l.c. The column (6 ft × 1/4 in. stainless steel) was packed with 20% DEGS on 60/80 AW firebrick, and the temperature was maintained at 150 °C.

Mass spectra were measured on a Nuclide 1290 DF double focusing, high-resolution mass spectrometer operated with a 4460 V accelerating potential and at 70 V electron energy. The high-resolution mass determinations were made with a peak matching system based on using perfluorokerosene as an internal standard. The samples were run in an all-glass inlet at 200 °C.

The n.m.r. spectra were measured in a Varian HA-100 spectrometer and the u.v. was recorded with a Cary 14 spectrophotometer.

The Organism

The fungus was isolated in August 1960 from frass taken from the inside of a dead deciduous tree on the Livingston Ranch near Walker, Louisiana. It is designated as NRRL 5161 in the ARS Culture Collection here, and is judged to be a strain of *Cephalosporium recifei* on the basis of the following cultural characteristics: colonies on wheat germ agar and Czapek agar were white with a faintly pink center; on synthetic mucor agar the colonies became golden yellow with a white margin, and on malt extract agar and potato dextrose agar their color was dull yellow–orange. Long tapering phialides typically formed on the substrate hyphae. On most media small synnema were present, from which phialides also arose. Phialides were usually single or branched, and less frequently were verticillate. Conidia were borne in moist, colorless heads and were typically ellipsoidal and curved. They measured (2) 3–7 μ (9) in length and 1–3 μ (4) in width. Because of the yellow color, the strain is assigned to the *Cephalosporium recifei* group, as described by Sukapure and Thirumalachar (5).

Production of Crude Lactone A

The stock culture of the fungus was maintained on YM

[1]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.

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 2% malt extract contained in 500-ml Erlenmeyer flasks, was inoculated with 0.1 ml of a cell suspension prepared from a 4-day-old YM slant culture and 1 ml of sterile distilled water. The flasks were incubated at 25 °C on a Gump shaker (180 r.p.m.; 1 in. radius). After 35 days growth the culture liquor was extracted with ether to give about 1.7 g of yellow viscous oil *A* per liter of medium.

*Isolation of Lactone of 11-Hydroxy-trans-8-dodecenoic Acid (**1**)*

Hexane extraction of oil *A* (10.0 g) at room temperature yielded 1.4 g of oil *B*, a portion (506 mg) of which was chromatographed on silicic acid (Bio-Sil A, 20 g), the eluting solvent being hexane–ether (95:5). The main fraction *C* (391 mg) was shown by g.l.c. to be 95% **1**; preparative g.l.c. of 167 mg *C* gave 88 mg of pure lactone of 11-hydroxy-*trans*-8-dodecenoic acid (**1**), a colorless mobile liquid, $[\alpha]_D^{25}$ +73.2° (*c*, 7.5, $CHCl_3$).

Anal. Calcd. for $C_{12}H_{20}O_2$: C, 73.43; H, 10.27. Found: C, 73.7; H, 10.4.

The u.v. spectrum: end absorption only. The i.r. spectrum (neat): 3010 (sh), 2978 (s), 2935 (s), 2850 (s), 1735 (s), 1450 (m), 1365 (m), 1225 (s), 975 cm^{-1} (s). The n.m.r. spectrum: doublet at δ 1.2 (methyl group attached to a carbon with one proton), 5.3 (two vinyl protons). Mass spectrum (Fig. 1): *m/e* 196 ($C_{12}H_{20}O_2$; found 196.1450, calcd. 196.1463).

*$KMnO_4$–HIO_4 Oxidation of 11-Hydroxy-trans-8-dodecenoic Acid (**3**)*

Fraction *C* (122 mg) refluxed 3 h with alcoholic KOH yielded 91 mg of hydroxy acid **3**, 47 mg of which was subjected to a von Rudloff (6) oxidation. The main product (26 mg) was shown by g.l.c. to consist of about 80% suberic acid. Identity was further established by conversion of 14 mg to the bisbenzhydrylamine salt (7) (28 mg; m.p. 172–174 °C), which was indistinguishable from an authentic sample.

*Ozonolysis of Lactone of 11-Hydroxy-trans-8-dodecenoic Acid (**1**)*

Ozone was passed for 45 s through a solution of fraction *C* (115 mg) in 5 ml ethanol and 1 ml ethyl acetate at 0 °C. The reaction mixture was taken to dryness, heated for 1 h at 95° with formic acid (98%, 3 ml) and H_2O_2 (30%, 0.2 ml), and kept overnight at room temperature. After additional heating (95 °C) for 35 min, the mixture was concentrated to 149 mg of oil, which was refluxed for 1 h with alcoholic KOH. Acidification and continuous ether extraction gave a first fraction of crude suberic acid (**9**) (44 mg; m.p. 133–138 °C). (Recrystallization: 26 mg, m.p. 140–142 °C; identity established by i.r. and mixture m.p.).

The second ether extract gave 65 mg of a semisolid. The third extract left 15 mg of oil ($[\alpha]_D^{25}$ −32° (*c*, 1, $CHCl_3$)), which was converted to the benzhydrylamine salt of **8** (m.p. 139–140 °C) the i.r. of which was identical with that of the corresponding salt prepared from *d,l*-3-hydroxybutyric acid.

*Hypoiodite Oxidation of 11-Ketododecanoic Acid (**6**)*

On hydrogenation (PtO_2; ethyl acetate), fraction *C* (101 mg) gave 67 mg of pure (g.l.c.) **2** (mass spectrum (Fig. 2): *m/e* 198 ($C_{12}H_{22}O_2$; found 198.1589; calcd. 198.1619). Saponification yielded 58 mg of hydroxy acid **5**, which was oxidized in 1 ml acetic acid with 0.56 ml of 0.53 *N* CrO_3–CH_3COOH for 1 h. When the resulting keto acid (57 mg) was subjected to the haloform reaction, it yielded 20 mg of iodoform, m.p. 123–124 °C. Ether extraction of the acidified reaction mixture gave 56 mg of solid, which g.l.c. analysis showed to be 48% 1,9-nonanedicarboxylic acid (**7**) and 62% unreacted 11-ketododecanoic acid (**6**).

*p-Bromophenacyl-11-hydroxy-trans-8-dodecenoate (**4**)*

11-Hydroxy-*trans*-8-dodecenoic acid (**3**) (18.5 mg) was converted to the *p*-bromophenacyl ester in acetone (15 min at 95°) by the dicyclohexylethylamine procedure (8). The crude product (22 mg; m.p. 60–68 °C) on crystallization from acetone–hexane gave 12 mg of *p*-bromophenacyl-11-hydroxy-*trans*-8-dodecenoate (**4**) (m.p. 73–74 °C).

Anal. Calcd. for $C_{20}H_{27}BrO_4$: C, 58.39; H, 6.61. Found: C, 58.5; H, 6.6.

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