Biological Oxidation of Grayanotoxin-II to the 3-Dehydro Derivative by a Microorganism

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Received July 5, 1976

Grayanotoxin (G)-II (1), $C_{20}H_{30}O_5$, mp 196~8°C, is one of the toxic diterpene alcohols obtained from *Leucothoe grayana* Max (Ericaceae).¹⁾ These alcohols are known for their novel carbon skeleton. We elucidated the biological oxidation of G-II (1) by a microorganism to give 3-dehydro G-II (2), $C_{20}H_{30}O_5$, mp 233°C.

MATERIALS AND METHODS

G-II (1) was isolated from the dried leaves of *Leucothoe grayana* Max in the manner described by S. Miyajima and S. Takei.^{1a)} The microorganism²⁾ (*Pseudomonas pseudomallei*) used was selected through growth tests on agar slants (2.0% agar and the basal medium, described in Table I, with 0.0025% G-II). The biological oxidation was carried out as below. G-II (1) was incubated with the microorganism at 30°C in the basal medium under aerobic conditions. The reaction period and the amounts of the substrate added are shown in Table II. Conditions are shown in the footnotes. Isolation of the products was carried out in the usual manner. After removing biomass by centri-

TABLE I. COMPOSITION OF THE MEDIUM

H ₂ O	1000 g	Na ₂ MoO ₄	0.0006 g
MgSO ₄ ·7H ₂ O	0.2	MnSO ₄	0.0006
CaCl ₂ ·2H ₂ O	0.1	Na ₂ HPO ₄ ·12H ₂ O	9.55
FeSO ₄ ·7H ₂ O	0.1	KH ₂ PO ₄	0.9
NH ₄ NO ₃	1.0	(pH 7.2~7.3)	

fugalization the culture broth was continuously extracted with ether and the extract was purified by successive crystallizations from ethyl acetate. When the extract contained the substrate, the product was isolated by preparative TLC (silica gel, C₆H₆:Et₂O:EtOH=5:2:0.5, 3 developments). Substances were detected as white bands on spraying water and extracted from silica gel with the mixed solvent of ethyl acetate and ethanol (3:1). Yields of the product are shown in Table II.

FIG. 1.

RESULTS AND DISCUSSION

On TLC analysis of the extracts, a clear spot of the main product (2) was detected at R_f 0.44 (2 developments). A faint spot was also observed at R_f 0.53 in process 1, and in process 4 faint spots appeared at R_f 0.34, 0.53, 0.64, 0.71, 0.81 and 0.93 from the residue of the mother liquid (2.5%). As shown in Table II, the activity of the microorganism decreased in processes 2 and 3. In process 3, the product (2) was detected on

TABLE II. CULTURE PERIOD AND YIELDS OF 3-DEHYDRO G-II

Process	G-II incubated		Period	Yields of 3-dehydro G-II		G-II recovered	
	mg			mg	(%)	mg	(%)
1	5	/	2 ^a	4.3	(86)	0	(0)
2	25	/	8 ^a	8.0	(32)	16	(64)
3	5	/	7 ^b	+	(0)	4.5	(90)
4	20	/	30 ^b	12.0	(60)	0	(0)

Microorganism: In process 1, the microorganism (100~150 mg, wet weight) used was grown on an agar slant similar to that used for cell selection. In processes 2 to 4, cells separated from the culture broth in the preceding process were used.

Medium: 100 ml.

Addition of G-II: G-II was added by the feeding method.

^a 2.5~3.0 mg/day. ^b 2.5 mg/3~4 days.

FIG. 2.

TLC analysis as a faint spot. In process 4, the activity was recovered and the substrate (1) was oxidized by the microorganism to give 3-dehydro G-II (2) (60% yield).

The main product was determined to be 3-dehydro G-II (2) from its spectral data. The product (2), $[\alpha]_D = -105^\circ$ ($c=0.23$, ethanol), showed the parent peak at m/e 350 ($C_{20}H_{30}O_6$) (6.8%) and several peaks at m/e 349 (10.5), 332 ($M^+ - H_2O$) (18.5), 315 (25.4), 314 ($M^+ - 2H_2O$) (100), 296 ($M^+ - 3H_2O$) (28.6), 286 (19.5), 285 (24.4), 175 (25.2), 149 (26.1) and 119 (42.8) on the MS spectrum. On IR spectrum (KBr), the product (2) showed absorptions due to hydroxyls (3600, 3520, 3450, 3380 cm^{-1}), a carbonyl (1735) and a terminal methylene (1646, 889). The NMR spectrum (Pyr. $-d_6 + D_2O$) showed signals due to three tertiary methyls (δ : 1.14, s, 1.47, s, 1.52, s), several methynes (δ : 4.46, s, $C_{14}-H$; 4.71, t, $J=6$, C_8-H) and a terminal methylene (δ : 5.09, s, 5.19, s). The spectrum was very similar to that of G-II (1), but lacked the signal corresponding to the C_3 -methine proton of G-II (1). Thus, the product was determined to be 3-dehydro G-II (2) and was identified with the authentic specimen⁸⁾ derived from

G-II (1) by its IR spectrum as shown in Fig. 2.

The reactivity of the secondary hydroxyl groups of G-II (1) toward acetylation was found to be in the decreasing order of C_8 -, C_6 - and C_{14} -hydroxyl group, but the reactivity toward hydrolysis showed the order of C_6 -, C_3 - and C_{14} -hydroxyl group.⁸⁾ In this case, it was found that the microorganism oxidized the most reactive hydroxyl group of G-II (1) toward acetylation.

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