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Biochemical Syntheses. II.[1] Microbial Transformation of Cyperotundone to Sugeonol and Isopatchoul-4-en-3-on-8α-ol[2]

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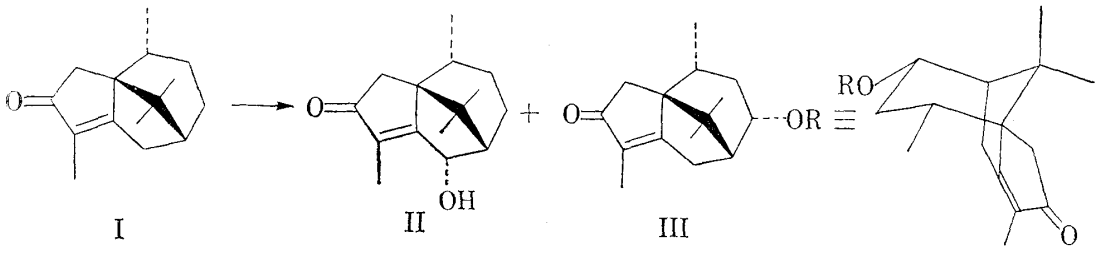
Cyperotundone (I), a constituent of nutgrass (*Cyperus rotundus* (Cyperaceae)), has been converted by exposure to *Corticium sasakii* into its oxygenated congeners, sugeonol (II), another constituent of nutgrass, and isopatchoul-4-en-3-on-8α-ol (III: R=H)) whose stereostructure has been deduced by its spectral properties.

During our work on the sesquiterpenic constituents of nutgrass, *Cyperus rotundus* Linné (Cyperaceae), of Japanese origin, we have isolated the ketone cyperotundone (I)[4] and later the keto-alcohol sugeonol, 6α-hydroxycyperotundone (II).[5] In the structural investigation of the latter (II), a few attempts for the conversion of cyperotundone (I) into sugeonol (II) by chemical procedure were made with the objective of obtaining a rigorous proof for the structure of the latter (II), but all were unsuccessful. As part of our study on the syntheses of natural products in biogenetical type by microorganisms, we have next initiated work directed towards a microbiological transformation of cyperotundone (I) into sugeonol (II). Thus in seeking 6α-hydroxylation of cyperotundone (I), it was noted that incubation with a number of organisms resulted in oxygenation of the substrate (I) judging by inspection of the vapor phase chromatograms and thin layer chromatograms of the products.

Fermentation of cyperotundone (I) with *Corticium sasakii*, the representative of the microorganisms, gave a product which by chromatographic examinations was shown to consist of mainly two components. Column chromatography of the product on silica gel yielded sugeonol (II), one of the main products, and a mixture of a number of more polar substances. Since separation of this mixture was impractical, the mixture was acetylated and chromatographed on silica gel yielding a keto-acetate (III: R=$COCH_3$). Isolation and characterization of the other fermentation products could not be achieved due to lack of the materials.



I II III

Hydrolysis of the acetate (III: R=$COCH_3$) afforded a free keto-alcohol (III: R=H), another main product. The molecular formulas ($C_{15}H_{22}O_2$ and $C_{17}H_{24}O_2$, respectively) of the ketol (III: R=H) and its acetate (III: R=$COCH_3$) suggest that the ketol is a monohydroxy-

1) Part I: *Tetrahedron*, **24**, 3147 (1968).
2) This paper forms Part XXIV in the series on Sesquiterpenoids. Preceding paper, Part XXIII: H. Hikino, H. Takahashi, Y. Sakurai, T. Takemoto, and N.S. Bhacca, *Chem. Pharm. Bull.* (Tokyo), **16**, 1081 (1968).
3) Location: *Kita-4-bancho, Sendai.*
4) H. Hikino, K. Aota, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **13**, 628 (1965); *ibid.*, **14**, 890 (1966).
5) H. Hikino, K. Aota, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 52 (1968).

lated derivative of cyperotundone (I). In accordance with this view, the infrared spectra (CCl_4) of the ketol (III: R=H) and the acetate (III: R=$COCH_3$) show the presence of hydroxyl (3650 and 3450 cm^{-1}) and an acetoxyl (1736 and 1236 cm^{-1}), respectively, as well as the retention of a cyclopentenone moiety (1705 and 1660 cm^{-1}, and 1709 and 1669 cm^{-1}, respectively). The nuclear magnetic resonance (NMR) spectra of the ketol (III: R=H) and the acetate (III: R=$COCH_3$) revealed the presence of one secondary methyl, two tertiary methyls, and one vinylic methyl in each molecule, demonstrating that the cyperotundone skeleton has still retained in this fermentation product (III) (Table I). The chemical shifts of the methyl signals

Table I

Compounds	Chemical shifts of the methyl protons (ppm)			
	C-12	C-13	C-15	C-14
Cyperotundone (I)	1.17	0.75	0.61	1.67
Isopatchoul-4-en-3-on-8α-ol (III: R=H)	1.08	0.79	0.63	1.67
8α-Acetoxyisopatchoul-4-en-3-one (III: R=$COCH_3$)	1.18	0.81	0.66	1.68

in the ketol (III: R=H) and its acetate (III: R=$COCH_3$) are also compatible with those in cyperotundone (I). The significant feature in the NMR spectra of both substances (III: R=H and $COCH_3$) are the signals arising from hydrogens on carbons bearing oxygen functions occurring at 4.15 and 5.15 ppm, indicating that the hydroxyl group introduced by fermentation is secondary and, therefore, must be situated at C-2, 6, 8, or 9 in the cyperotundone skeleton. However, the splitting patterns of the signals (a doublet of doublets of doublets) exclude the possible location of the hydroxyl group at C-2 or C-6. If the hydroxyl group were oriented at C-8α or C-8β position, the coupling constants are calculated as 3—6, 3—8, and 16—11 cps or 3—1, 2—0, and 4—7 cps, respectively, from inspection of Dreiding models, and from the dihedral angles by the Williamson-Johnson version of the Karplus equation.[6] On the other hand, if the hydroxyl group were situated at C-9α or C-9β position, the coupling constants are known to be 4—5, ~0, and 4—5 cps[7] or 10, 7, and 10 cps,[8] respectively. In reality, the observed coupling constants for the carbinyl hydrogen signals (J=3—4, 6, 11 cps) point to the situation of the hydroxyl group at C-8α position. On the basis of the above evidence the by-product (III: R=H) is shown to be isopatchoul-4-en-3-on-8α-ol.

In conclusion, the selective 6α-hydroxylation of cyperotundone, which must be effected as part of biosynthetic pathways of the constituents in nutgrass, could be reproduced by the microorganism, *Corticium sasakii*.

Experimental[9]

Fermentation of Cyperotundone with *Corticium sasakii* ——*Corticium sasakii*[10] was grown in a modified Corticium synthetic medium[11] (2 liter in twenty 500 ml flasks) at 27° on a reciprocal shaker. After 4 days of incubation, cyperotundone (I) (0.60 g) dissolved in EtOH (20 ml) was distributed equally among the 20 flasks. The fermentation was continued at 27° for 4 days. The culture broth then was extracted with AcOEt, and the extract evaporated to dryness. The residue (1.04 g) was chromatographed on silica gel (20 g).

6) K.L. Williamson and W.S. Johnson, *J. Am. Chem. Soc.*, **83**, 4623 (1961).
7) H. Hikino, K. Ito, K. Aota, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 43 (1968).
8) H. Hikino, K. Aota, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **15**, 1433 (1967).
9) NMR spectra were recorded at 60 Mcps. Chemical shifts are given in ppm downward from internal Me_4Si, and coupling constants (J) in cps. Abbreviations: s=singlet, d=doublet, and t=triplet.
10) The culture of the microbe was supplied by Institute for Fermentation, Osaka, to whom we express our thanks.
11) H. Hikino, Y. Tokuoka, Y. Hikino, and T. Takemoto, *Tetrahedron*, **24**, 3147 (1968).

The fraction (88 mg) eluted with benzene–AcOEt (5:1) was crystallized from ether to give sugeonol (II) as colorless needles, mp 181—182.5° (uncorr), $[\alpha]_D$ +68.9° (c=2.9, $CHCl_3$). *Anal.* Calcd. for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46. Found: C, 76.79; H, 9.50. IR ($CHCl_3$) cm^{-1}: 3640, 3430 (hydroxyl), 1696, 1660 (cyclopentenone), 1416 (methylene adjacent to carbonyl). Identity was confirmed by the usual criteria.

The fraction (115 mg) eluted with benzene–AcOEt (2:1) was dissolved in pyridine (0.8 ml) and treated with Ac_2O (0.5 ml) at room temperature overnight. Upon isolation in the customary manner, the product (119 mg) was chromatographed over silica gel (2 g). Elution with benzene gave an oil (51 mg) which on distillation under reduced pressure afforded 8α-acetoxyisopatchoul-4-en-3-one (III: R=$COCH_3$) as a colorless oil, $[\alpha]_D$ −17.0° (c=2.8, $CHCl_3$). *Anal.* Calcd. for $C_{17}H_{24}O_3$: C, 73.88; H, 8.75. Found: C, 73.84; H, 8.89. IR (CCl_4) cm^{-1}: 1736, 1236 (acetoxyl), 1709, 1669 (cyclopentenone), 1418 (methylene next to carbonyl), NMR (CCl_4): 3H d at 0.66 (J=6, $C_{(15)}\underline{H}_3$), 3H s at 0.81 ($C_{(13)}\underline{H}_3$), 3H s at 1.18 ($C_{(12)}\underline{H}_3$), 3H t at 1.68 (J=1, $C_{(14)}\underline{H}_3$), 3H s at 1.97 ($C\underline{H}_3$–COO–), 1H ddd at 5.15 (J=3, 6, 11, $C_{(8\beta)}\underline{H}$).

To the acetate (III: R=$COCH_3$) (43 mg) in EtOH (1 ml) was added ethanolic KOH solution (0.6%, 2 ml), and then the mixture was stirred at room temperature for 2.5 hr. Isolation in the usual way and distillation under diminished pressure furnished isopatchoul-4-en-3-on-8α-ol (III: R=H) as a colorless oil, $[\alpha]_D$ +9.7°(c=4.1, $CHCl_3$). *Anal.* Calcd. for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46. Found: C, 76.65; H, 9.43. IR (CCl_4) cm^{-1}: 3650, 3450 (hydroxyl), 1705, 1660 (cyclopentenone), 1416 (methylene α to carbonyl). NMR (CCl_4): 3H d at 0.63 (J=7, $C_{(15)}\underline{H}_3$),3H s at 0.79 ($C_{(13)}\underline{H}_3$), 3H s at 1.08 ($C_{(12)}\underline{H}_3$), 3H t at 1.67 (J=1, $C_{(14)}\underline{H}_3$), 1H ddd at 4.15 (J=4, 6, 11, $C_{(8\beta)}\underline{H}$).

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