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Microbial Transformation of Sesquiterpenoids. V.[1] Preparation of 1-Epiliguloxide, the Sixth Stereoisomer of Guaioxide

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Microbial hydroxylation of guaioxide has played an important role in establishing the stereochemistry of the compound[3] and its diastereomers.[1,4] Thus, out of the eight possible stereoisomers (I—VIII) of guaioxide involving the asymmetric centers at C-1, C-4 and C-10, five compounds have so far been characterised; they are guaioxide (I),[3,5] 4-epiguaioxide (II, liguloxide),[4] 10-epiguaioxide (III, bulnesoxide),[1,6] 10-epiliguloxide (IV)[4] and 1-epiguaioxide (V).[7]

We have undertaken preparation of the sixth stereoisomer, 1-epiliguloxide (VI), by use of microbial oxidation of liguloxide (II), since the naturally occurring compound II was available to us[4] as the starting material. Further, we were interested in the comparison of 1-epiliguloxide with a compound tentatively named LB, which was isolated by Tanahashi, *et al.*[8] from a Chinese crude drug "San-shion" and shown to be an isomer of guaioxide other than one of the five compounds (I—V).

We found that *Streptomyces purpurescens* oxidized liguloxide (II) to produce the 2α-hydroxy derivative (IX), a compound suitable for our purpose, along with the known[9] 3α-hydroxy-, 8α-hydroxy-, 8β-hydroxy- and 9α-hydroxyliguloxides.

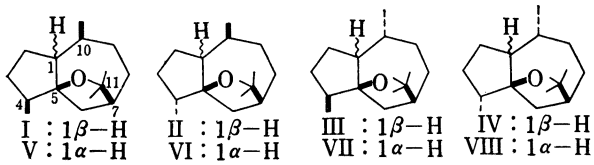

Chart 1

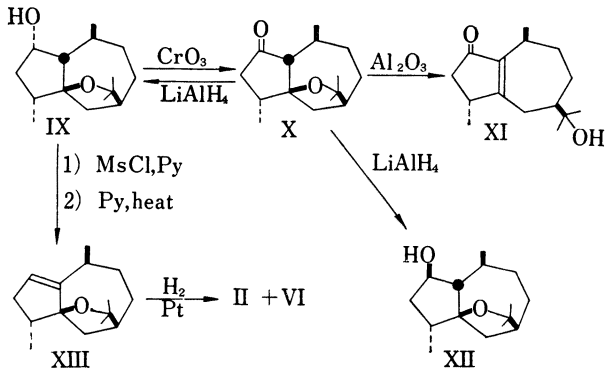

Chart 2

1) Part IV: H, Ishii, T. Tozyo and M. Nakamura, *Tetrahedron*, **27**, 4263 (1971).
2) Location: *Fukushima-ku, Osaka*; *a*) Present address: *Badische Anilin und Soda-Fabrik AG, Ludwigshafen/Rhein, Germany.*
3) H. Ishii, T. Tozyo, M. Nakamura and H. Minato, *Tetrahedron*, **26**, 2751 (1970).
4) H. Ishii, T. Tozyo, M. Nakamura and H. Minato, *Tetrahedron*, **26**, 2911 (1970).
5) R.B. Bates and R.C. Slagel, *Chem. Ind.* (London), **1962**, 1715.
6) P. Ledoux, B.P. Vaterlaus and G. Chiurdoglu, *Bull. Soc. Chim. Belges*, **75**, 551 (1966).
7) C. Ehret and G. Ourisson, *Bull. Soc. Chim. France*, **1968**, 2629.
8) Y. Tanahashi, S. Tomoda and T. Takahashi, *Bull. Chem. Soc. Japan*, **42**, 2076 (1969).
9) E. Funke, T. Tozyo, H. Ishii and K. Takeda, *J. Chem. Soc.* (*C*), **1970**, 2548.

The new product (IX), mp 99—100°, $C_{15}H_{26}O_2$, showed an infrared (IR) band at 3430 cm^{-1} (OH) and on Jones oxidation it gave a five-membered ring ketone (X), ν_{max} 1744 cm^{-1}. When passed through a column of alumina compound X underwent opening of the ether bridge to give an α,β-unsaturated ketone (XI), ν_{max} 1702 and 1640 cm^{-1}, indicating that the carbonyl group in X is present on C-2 or C-3.[1,9] The nuclear magnetic resonance (NMR) spectrum of XI showed two doublet methyl signals at δ 1.02 (3H, J=7 Hz) and 1.18 (3H, J=7 Hz), excluding the Δ^4-3-oxo-structure for the compound.[9] Hence the carbonyl group in XI and thus in X must be present at C-2. Optical rotatory dispersion (ORD) data for the ketone (X) [negative Cotton effect (a −227)] also agreed with a 2-oxo- rather than a 3-oxo-structure.[10]

Lithium aluminum hydride reduction of X gave a 3:2 mixture of the original alcohol (IX) and its epimer (XII). The configuration of the C-2 hydroxyl groups in these alcohols was determined by application of the benzoate rule.[11] The $\Delta[M]_D$ value for the product IX series was +97°, whereas the value for the epimer XII series was −166°. Thus compound IX has the 2(S)-configuration (2α-OH) and its epimer the 2(R)-configuration (2β-OH).

2α-Hydroxyliguloxide (IX) was treated with mesyl chloride in pyridine at room temperature and the crude mesylate thus obtained was heated with pyridine to give a dehydrated compound (XIII) as the main product. Since its NMR spectrum showed a signal at δ 5.33 (1H, m) due to one vinyl proton, the structure 1-dehydroliguloxide was assigned to XIII. Catalytic reduction of XIII without hydrogenolytic cleavage of the allylic ether linkage was achieved by hydrogenation over Adams catalyst in a 4:1 mixture of ethyl acetate and acetic acid, giving a 10:1 mixture of liguloxide (II) and its 1-epimer (VI). Both compounds were separated from the mixture by silica gel chromatography. 1-Epiliguloxide (VI) is a colorless oil, $[\alpha]_D$−25.6°, which showed a mass spectrum (M^+ 222) identical with that of liguloxide.

Comparisons of the IR and NMR spectra of this oxide (VI) and LB clearly indicated the dissimilarity of the compounds. Thus the structure of LB remains unknown.

Experimental

Rotations were taken for solutions in dioxane. Unless otherwise stated, NMR spectra were recorded on a Varian A-60 spectrometer for solutions in $CDCl_3$ using TMS as internal standard.

Fermentation of Liguloxide (II)——Liguloxide (II, 1.9 g) was fermented with *Streptomyces purpurescens* for 4 days as described for the fermentation of guaioxide.[1] The filtrate of the culture broth was extracted with $CHCl_3$–MeOH (1:1) and the extract was evaporated leaving a brown oil (1.424 g). The oil was distilled at up to 180° (bath)/3 mm to give a viscous oil (544 mg), which was chromatographed on silica gel (30 g) and eluted successively with petr. ether and petr. ether–ether (95:5, 9:1, 4:1 and 1:1). The tail eluate with petr. ether and the eluate with petr. ether–ether (95:5) were combined to yield, after removal of the solvent, the starting material (II. 241 mg).

The eluate with petr. ether–ether (4:1) gave 2α-hydroxyliguloxide (IX) as colorless plates (83 mg), mp 99—100° (sublimation), $[\alpha]_D^{26}$ −59.2° (c=1.079). *Anal.* Calcd. for $C_{15}H_{26}O_2$: C, 75.58; H, 11.00. Found: C, 75.32; H, 10.93. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH). NMR δ: 0.96 (3H, d, J=5.8 Hz, 10-Me), 1.00 (3H, d, J=6.5 Hz, 4-Me), 1.15 and 1.30 (each 3H, s, 11-Me_2), and 4.35 (1H, m, 2-H); δ (C_5D_5N): 1.07 (3H, d, J=6 Hz, 10-Me), 1.10 (3H, d, J=6.5 Hz, 4-Me), 1.15 and 1.32 (each 3H, s, 11-Me_2), and 4.35 (1H, m, 2-H). The benzoate was a colorless oil, $[\alpha]_D^{22}$ −12.8° (c=0.619). *Anal.* Calcd. for $C_{22}H_{30}O_3$: C, 77.15; H, 8.83. Found: C, 77.18: H, 8.79.

The eluate with petr. ether–ether (1:1) (175 mg) was separated into six fractions by preparative thin-layer chromatography (TLC) [silica gel; benzene–EtOHc (4:1) (two development); Morin-UV]. The second fastest running fraction gave 8α-hydroxyliguloxide as an oil (24 mg), identical with an authentic sample.[9] The third, fourth, and fifth fastest running fractions also gave the known[9] 3α-hydorxyliguloxide (an oil, 59 mg), 8β-hydroxyliguloxide (needles, mp 138—140°, 17 mg) and 9α-hydroxyliguloxide (needles, mp 113—115°, 25 mg), respectively.

10) W. Klyne, *Tetrahedron*, **13**, 29 (1961).
11) J.H. Brewster, *Tetrahedron*, **13**, 106 (1961).

2-Oxoliguloxide (X)——Jones reagent (0.1 ml) was added to a cooled solution of IX (35 mg) in acetone (1 ml) and the mixture was stirred for 3 min at room temperature. The reaction mixture was worked up in the usual manner to give an oil (34 mg), which was distilled at 110° (bath)/4 mm yielding X (30 mg) as a colorless oil, $[\alpha]_D^{22}$ −196.1° (c=0.609). *Anal.* Calcd. for $C_{15}H_{24}O_2$: C, 76.22; H, 10.24. Found: C, 75.96; H, 10.22. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1744 (C=O). NMR δ (C_6D_6): 0.85 (3H, d, J=6 Hz, 4-Me), 1.02 and 1.13 (each 3H, s, 11-Me_2), and 1.26 (3H, d, J=6.5 Hz, 10-Me). ORD (c=0.609 in dioxane): $[\theta]_{450}$ −138°, $[\theta]_{326}$ −12337°, $[\theta]_{280}$+10439°, $[\theta]_{235}$+5504°.

Rearrangement of X on Alumina——A solution of X (15 mg) in petr. ether (2 ml) was placed on a column of Al_2O_3 (5 g, activity I) and left for 3 hr. Elution with ether gave the product XI as an oil (14 mg), $[\alpha]_D^{22}$ −43.1° (c=0.568). *Anal.* Calcd. for $C_{15}H_{24}O_2$: C, 76.22; H, 10.24. Found: C, 76.02; H, 10.27. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3615, 3500 (OH), 1702, 1640 (C=C–C=O). UV λ_{max}^{EtOH} mμ (ε): 241.5 (13500) (C=C–C=O). NMR δ: 1.02 (3H, d, J=7 Hz) and 1.18 (3H, d, J=7 Hz) (4-Me and 10-Me), and 1.26 (6H, s, 11-Me_2).

2α-Hydroxyliguloxide (XII)——A solution of X (120 mg) in dry ether (5 ml) was added dropwise to a suspention of $LiAlH_4$ (100 mg) in dry ether (3 ml) and the mixture was stirred at room temperature for 1 hr. The excess of the reagent was decomposed by addition of H_2O and the mixture was extracted with $CHCl_3$. The extract was washed, dried (Na_2SO_4) and evaporated to give a pale yellow oil (115 mg). The oil was divided into three fractions by preparative TLC [silica gel; benzene–EtOAc (4:1); Morin-UV]. The fraction having the highest mobility gave 2α-hydroxyliguloxide (IX), mp 99—100° (54 mg), identical with the authentic sample described above.

The fraction having the lowest mobility afforded 2β-hydroxyliguloxide (XII), an oil (35 mg), $[\alpha]_D^{22}$ −73.2° 0.526). *Anal.* Calcd. for $C_{15}H_{26}O_2$: C, 75.58; H, 11.00. Found: C, 75.27; H, 10.88. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3605, 3440 (OH). NMR δ: 0.95 (3H, d, J=6.5 Hz, 4-Me), 1.05 (3H, diffuse d, 10-Me), 1.15 and 1.30 (each 3H, s, 11-Me_2), and 3.67 (1H, m, 2-H); δ (C_5D_5N): 0.97 (3H, d, J=6.5 Hz, 4-Me), 1.22 (3H, diffuse d^6, 10-Me), 1.13 and 1.30 (each 3H, s, 11-Me_2), and 3.87 (1H, m, 2-H). The benzoate was an oil, $[\alpha]_D^{22}$ −99.5° (c=0.566). *Anal.* Calcd. for $C_{22}H_{30}O_3$: C, 77.15; H, 8.83. Found: C, 77.21; H, 8.76.

1-Dehydroliguloxide (XIII)——Mesyl chloride (0.5 ml) was added to a cooled solution of IX (620 mg) in pyridine (6 ml) and the mixture was set aside at room temperature for 1 hr. The mixture was poured into ice-water, which was then extracted with $CHCl_3$. The extract was washed successively with dil. H_2SO_4, 5% Na_2CO_3 aq. and H_2O, dried (Na_2SO_4) and evaporated to give a brown oil (924 mg). The oil was chromatographed on silica gel (30 g) and eluted with petr. ether–ether (95: 5, 9: 1 and 4:1). The fraction eluted with petr. ether–ether (4: 1) gave a crystalline substance (600 mg), which was dissolved in pyridine (10 ml) and heated at 100° for 2 hr. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed, dried (Na_2SO_4) and evaporated leaving a brown oil (271 mg). The oil was chromatographed on silica gel (10 g) and eluted with petr. ether–ether (99: 1, 98: 2, 95: 5 and 9:1). The eluate from petr. ether–ether (98: 2) gave 1-dehydroliguloxide (XIII) as an oil (104 mg). NMR δ: 0.88 (3H, d, J=7 Hz, 4-Me), 1.13 (3H, d, J=7 Hz, 10-Me), 1.15 and 1.26 (each 3H, s, 11-Me_2), and 5.33 (1H, m, 2-H).

Catalytic Hydrogenation of XIII——A mixture of XIII (104 mg) and Adams catalyst (30 mg) in a mixture of AcOH (2 ml) and EtOAc (8 ml) was hydrogenated at room temperature. When 1.05 mole H_2 had been absorbed, the reaction ceased, and the catalyst and solvent were removed. The residue (105 mg) aws chromatographed on silica gel (20 g) and eluted with petr. ether–ether (99: 1). The head eluate gave 1-epiliguloxide (VI) as a colorless oil (9 mg), $[\alpha]_D^{21}$ −25.6° (c=0.199). Mass Spectrum m/e: 222 (M^+). NMR δ: 0.90 (3H, diffuse d, 10-Me), 0.98 (3H, d, J=6.5 Hz, 4-Me), 1.15 and 1.30 (each 3H, s, 11-Me_2).

The tail eluate afforded liguloxide (II), mp 36° (90 mg), identical with an authentic sample.

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