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MICROBIAL TRANSFORMATION OF SESQUITERPENOIDS—I

STRUCTURE ELUCIDATION OF GUAIOXIDE WITH THE AID OF MICROBIAL HYDROXYLATION[1]

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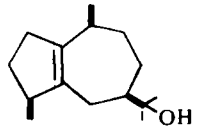
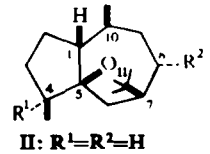
Abstract—Guaioxide is a saturated sesquiterpene oxide, for which four possible structures have been suggested. Attempted microbial hydroxylation of the compound was successful, this led to a way to open its oxide linkage under mild conditions, and to a decision of the stereochemistry II for guaioxide.

GUAIOXIDE is a minor sesquiterpene component of guaiac wood oil.[2] It is also obtained by acid-catalysed cyclisation [2-4] of guaiol (I), the stereochemistry of which is known,[5] so that there have been suggested four possible structures: two of these have a terminal of the ether linkage at C-1 and the other two at C-5. We have recently isolated liguloxide[6] from *Ligularia fischeri* Turcz and found that this compound contains a similar carbon-oxygen framework to that of guaioxide. In connection with structure elucidation of liguloxide, we have studied the structure of guaioxide and decided its stereochemistry as II.

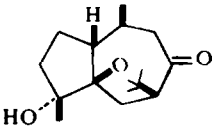
To develop a way to open the ditertiary ether linkage in guaioxide under mild conditions, we have used the microbial oxidation technique as a means of introducing hydroxyl groups into this saturated sesquiterpene oxide. After making several screening tests using thirty kinds of microorganisms, we selected *Mucor parasiticus* Bain



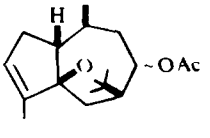
I



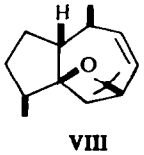
II: R^1=R^2=H
IIIa: R^1=R^2=OH
IIIb: R^1=OH, R^2=OAc
IVa: R^1=H, R^2=OH
IVb: R^1=H, R^2=OAc
V: R^1=OH, R^2=H



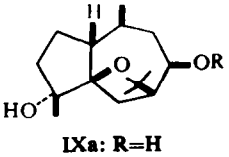
VI



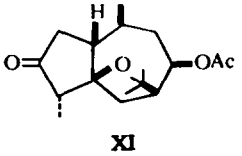
VII: 8α-OAc
X: 8β-AOc



VIII



IXa: R=H
IXb: R=Ac



XI

XIIa: R=H
XIIb: R=Ac

CHART 1

(ATCC 6476) because it oxidized guaioxide to produce three hydroxylated compounds (IIIa, IVa and V) as main products.

One of the products (IIIa), m.p. 172–173°, proved to be a dihydroxylated guaioxide from its elemental analysis ($C_{15}H_{26}O_3$). Its NMR spectrum showed three singlet methyl signals at τ 8·77, 8·73 and 8·71, indicating that one of the hydroxyl groups introduced must be located on the carbon bearing a methyl group (C-4 or C-10). Jones oxidation of IIIa gave a hydroxy-ketone (VI), the IR spectrum of which displayed a carbonyl band at 1688 cm^{-1}, showing that the secondary hydroxyl group introduced should be present on either C-6, C-8, or C-9. That these two hydroxyl groups are located on C-4 and C-8 was inferred in the course of the interrelation of two products (III and IV) with guaioxide (II).

The hydroxy-acetate (IIIb) was treated with thionyl chloride in pyridine to give a dehydrated product (VII), which was catalytically hydrogenated to form a 1:1 mixture of the acetate (IVb) and its C-4 epimer (see below). Since the former was identical with the acetate of the second product (IVa), $C_{15}H_{26}O_2$, m.p. 82–83°, the acetylable hydroxyl groups in the two products (IIIa and IVa) must occur at the same positic n. Dehydration of the second product (IVa) with mesyl chloride and pyridine gave only one product (VIII), its NMR spectrum showing the presence of a —CH=CH— grouping (τ 4·57, 2H) in the molecule. Because the retention of the guaioxide skeleton in VIII was confirmed by regeneration of guaioxide (II) on catalytic hydrogenation of VIII, the structure of compound VIII was clarified as 8 dehydroguaioxide. Therefore, taking Bredt's rule into account, we assumed that both the microbial hydroxylation products (IIIa and IVa) must carry a C-8 hydroxyl group, and hence that the tertiary hydroxyl group in IIIa must be located on C-4 since stability of the hydroxy-ketone (VI) to alkaline treatment ruled out its location on C-10. Based upon these assumptions, an attempt to correlate compound IIIa with torilolone of established structure (XIIa)[7] was successfully made.

Reduction of the hydroxy-ketone (VI) with LAH produced predominantly an epimeric diol (IXa), in which the hydroxyl group on C-8 seemed to have the same β-configuration as that in torilolone (XIIa),[7] since it is reasonable to consider that attack of the reagent on C-8 should occur from the less hindered α-side (the side opposite to the ether bridge). Dehydration of its mono-acetate (IXb) with thionyl chloride in pyridine afforded an unsaturated acetate (X), which carried a —CH=CMe-grouping (τ 8·33, 3H, and 4·60, 1H). Hydroboration followed by Jones oxidation changed compound X into a keto-acetate (XI), ν_{max} 1745cm^{-1} (five-membered ring ketone and OAc), the absorption verified that the keto group in XI is located on C-3, and also that the tertiary hydroxyl group in IIIa is on C-4.

As the C-3 oxo group in XI is situated in a β-position towards either C-1 or C-5, alkaline treatment of the ketone (XI) is expected to bring about β-elimination of the ether linkage on these positions as well as simultaneous formation of a double bond (Δ^1 or Δ^4); from the NMR signals due to the double bond we should be able to decide the terminal position (C-1 or C-5) of the ether linkage in XI and thus in guaioxide. In fact chromatography of the C-3 oxo compound (XI) on alumina resulted in the formation of a Δ^4-compound, *i.e.*, torilolone acetate (XIIb), its structure being confirmed by comparison with an authentic sample prepared by acetylation of torilolone.[7] This established that the ethereal oxygen atom in the guaioxide skeleton is linked to C-5 and not to C-1.

Correlation of the product (IIIa) and torilolone (XIIa) also showed that the C-8 hydroxyl group in IIIa is α-oriented, the hydroxyl group having been epimerized during the series of reactions. A decision on the configuration of the C-4 hydroxyl group in IIIa was possible from the IR measurement: the IR spectrum of IIIa taken in dilute solution (0·068% in CCl_4) revealed a hydroxyl absorption at 3620 cm^{-1}, suggesting the absence of intramolecular hydrogen bonding between the 5β-ethereal oxygen and the C-4 hydroxyl group. This showed that the hydroxyl group is α-oriented, and that the product (IIIa) is 4α,8α-dihydroxyguaioxide.

Since the second product has been derived from IIIa as described above, it is identified as 8α-hydroxyguaioxide (IVa). The last product, $C_{15}H_{26}O_2$, a colorless oil, was prepared by Huang–Minlon reduction of 4α-hydroxy-8-oxoguaioxide (VI), indicating it to be 4α-hydroxy-guaioxide (V).

The original configuration of the C-4 methyl group in guaioxide is most likely to be β, because microbial hydroxylation is generally accepted to proceed with retention of the configuration at the position being hydroxylated.[8] That this assignment is correct has recently been confirmed by Tanahashi *et al.* from another point of view.[9]

The remaining problem is the stereochemistry at C-1 in guaioxide. ORD measurements on the ketone (XI) showed a strong positive Cotton effect curve (a = + 267). Inspection of a molecular model of the compound and application of the octant rule[10] indicate that the configuration at C-1 in XI is β, and from the very large amplitude, the orientation of the C-4 methyl group is assumed to be α. It is clear that inversion at C-1 cannot occur in the course of transformation from guaioxide to XI, so guaioxide should be represented by the stereochemistry II.

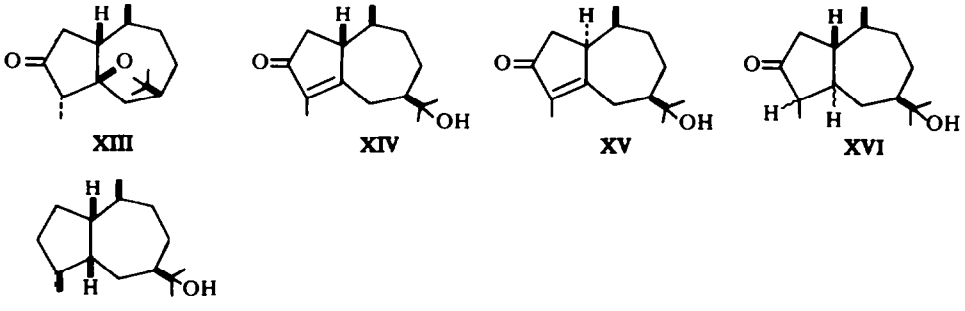


CHART 2

Chemical proof of the β-configuration of C-1 was obtained by the conversion of 3-oxo-4-epiguaioxide (XIII) into the known 1β, 5β-dihydroguaiol (XVII).[11, 12] Compound XIII was prepared from 4α-hydroxyguaioxide (V) by a similar sequence of reactions to that for the dihydroxyguaioxide (III) series. Chromatography of XIII on alumina gave 8-deoxytorilolone (XIV), which on heating with 3% methanolic potassium hydroxide yielded a 1 : 1 equilibrium mixture of XIV and its C-1 epimer (XV). Since both the compounds were stable on alumina chromatography, and XIV was obtained as a sole product from the ketone (XIII), the configuration at C-1 of XIV must be the same as that of XIII, and thus that of guaioxide. 8-Deoxytorilolone (XIV)

was hydrogenated to give a 1:1 mixture of two dihydro-derivatives (XVI), isomers of *cis*- and *trans*-ring junctions. Huang–Minlon reduction of the mixture afforded a mixture of two deoxo-derivatives (1:1), which were separated by preparative GLC. One of them proved to be identical with an authentic sample of 1β, 5β-dihydroguaiol (XVII), prepared by high-pressure hydrogenation[13] of guaiol (I) followed by GLC separation.

This structure confirmation of guaioxide has played an important role in the structure determination of liguloxide,[6] which is now known as 4-epiguaioxide.

EXPERIMENTAL

Rotations were taken in dioxane unless otherwise noted. NMR spectra were recorded on a Varian A-60 spectrometer in $CDCl_3$ with TMS as internal standard with $\tau = 10$. Coupling constants are expressed in c/s. GLC was run using an Aerograph Autoprep model A-700 instrument.

Fermentation of guaioxide (II) *with* Mucor parasiticus. Two hundred 500 ml shaking flasks, each containing 100 ml of sterilized nutrient soln composed of 4% glucose, 2% peptone and 0·3% corn steep liquor were inoculated with *Mucor parasiticus* Bain (ATCC 6476). After shaking at 27° for 24 hr, a soln of guaioxide (*65 mg*) in acetone (1 ml) was added to each flask and the fermentation was continued at 27° for a further 2 days. The filtrate of the culture broths was extracted with $CHCl_3$ and the extract was evaporated leaving a fermentation product (14·9 g).

The product was chromatographed on alumina (activity V, 500 g) and eluted successively with light petroleum-ether (99:1, 98:2, 95:5, 9:1, 4:1 and 1:1) and ether. The head eluate with light petroleum-ether (9:1) gave a crude product (V). Repeated chromatography of the crude product yielded a pure sample of 4α-hydroxyguaioxide (V) as a colorless oil (400 mg), $[\alpha]_D^{22}$ −35·4° (± 0·8°) (*c* 0·908); IR ($CHCl_3$) 3600 cm^{-1} (OH); NMR 9·13 (3H, diffused d) [Me on C-10], 8·82 (3H, s) and 8·69 (6H, s) [Me on C-4 and C-11] (Found: C, 75·49; H, 11·03. $C_{15}H_{26}O_2$ requires C, 75·58; H, 11·00%). The tail eluate with light petroleum-ether (9:1) afforded 8α-hydroxyguaioxide (IVa) as colorless prisms (from n-pentane, 1·015 g), m.p. 82–83°, $[\alpha]_D^{22}$ −23·0° (± 0·6°) (*c* 1·103); IR ($CHCl_3$) 3605 and 3445 cm^{-1} (OH); NMR 9·08 (3H, d, $J = 6$) and 9·03 (3H, d, $J = 6·5$) [Me on C-4 and C-10], 8·81 (3H, s) and 8·72 (3H, s) [two Me on C-11], and 5·93 (1H, m) [H on C-8] (Found: C, 75·46; H, 10·93. $C_{15}H_{26}O_2$ requires C, 75·58; H, 11·00%). The eluate with ether yielded 4α, 8α-dihydroxyguaioxide (IIIa) as colorless plates (from ether, 1·147 g), m.p. 172–173°, $[\alpha]_D^{23}$ −34·1° (± 0·8°) (*c* 0·959); IR ($CHCl_3$) 3600 and 3450 cm^{-1} (OH); NMR 9·00 (3H, diffused d) [Me on C-10], 8·77 (3H, s), 8·73 (3H, s) and 8·71 (3H, s) [Me on C-4 and C-11], and 5·93 (1H, m) [H on C-8] (Found: C, 70·55; H, 10·25. $C_{15}H_{26}O_3$ requires C, 70·83; H, 10·30%).

Acetylation of 4α,8α-*dihydroxyguaioxide* (IIIa). To a soln of IIIa (100 mg) in pyridine (1 ml), Ac_2O (0·5 ml) was added and the mixture was set aside overnight at room temp. Working up in the usual manner and sublimation of the product gave 8α-acetoxy-4α-hydroxyguaioxide (IIIb) as colorless prisms, m.p. 69–71°; IR (Nujol) 3520 (OH), 1715 and 1240 cm^{-1} (OAc) (Found: C, 68·61; H, 9·44. $C_{17}H_{28}O_4$ requires C, 68·89; H, 9·52%).

Jones oxidation of 4α,8α-*dihydroxyguaioxide* (IIIa). Jones reagent (0·2 ml) was added to a cooled soln of IIIa (100 mg) in acetone (1 ml) and the mixture was stirred for 5 min at room temp. The reaction mixture was worked up in the usual manner to give a crystalline substance (95 mg), which was recrystallized from light petroleum yielding 4α-hydroxy-8-oxoguaioxide (VI) as colorless prisms, m.p. 72–74°, $[\alpha]_D^{22}$ + 93·6° (± 1·3°) (*c* 1·033); IR ($CHCl_3$) 3600 (OH) and 1688 cm^{-1} (CO); NMR 9·05 (3H, diffused d) [Me on C-10] and 8·72 (6H, s) and 8·62 (3H, s) [Me on C-4 and C-11] (Found: C, 71·40; H, 9·58. $C_{15}H_{24}O_3$ requires C, 71·39; H, 9·59%).

Conversion of 8α-*acetoxy-4α-hydroxyguaioxide* (IIIb) *into* 8α-*acetoxyguaioxide* (IVb). Thionyl chloride (0·1 ml) was added to a soln of IIIb (110 mg) in pyridine (1 ml), and the mixture was left for 2·5 hr at room temp. Ice–water was added to the reaction mixture, which was extracted with $CHCl_3$. The extract was washed, dried (Na_2SO_4) and evaporated leaving an oil (105 mg), which was chromatographed on alumina (activity V, 10 g). Elution with n-pentane gave 8α-acetoxy-3-dehydroguaioxide (VII) as a viscous oil (77 mg), IR (film) 1730, 1240 (OAc) and 1657 cm^{-1} (—CH=C<); NMR 9·10 (3H, d, J=

6) [Me on C-10], 8·75 (3H, s) and 8·63 (3H, s) [two Me on C-11], 8·32 (3H, d, $J = 1·2$) [Me on C-4], 7·97 (3H, s) [OAc], 4·83 (1H, broad) [H on C-8], and 4·60 (1H, m) [H on C-3]. A mixture of VII (55 mg) and Adams' catalyst (25 mg) in AcOH (5 ml) was hydrogenated at room temp. The oily product (54 mg), which showed two peaks of retention times 8·0 min (53%) and 11·1 min (47%) on GLC (5% Diethyleneglycol succinate; 170°; He 200 ml/min), was chromatographed on alumina (activity IV, 10 g). The head eluate with n-pentane gave 8α-acetoxyguaioxide (IVb) as colorless prisms (sublimation, 14 mg, retention time 8·0 min), m.p. 56–57°, $[\alpha]_D^{20}$ —25·6° (± 1·0°) (*c* 0·660); IR (Nujol) 1728, 1250 and 1230 cm^{-1} (OAc); NMR 9·09 (3H, d, $J = 6$) and 9·01 (3H, d, $J = 6·5$ [Me on C-4 and C-10], 8·81 (3H, s) and 8·86 (3H, s) [two Me on C-11], 7·94 (3H, s) [OAc], and 4·86 (1H, m) [H on C-8] (Found: C, 72·89; H, 9·97. $C_{17}H_{28}O_3$ requires C, 72·82; H, 10·06%). This was identical with a sample prepared by acetylation of IVa (IR spectrum and mixed m.p.).

The tail eluate with n-pentane gave a mixture of both compounds (26 mg), and the eluate with n-pentane-ether (98·2) furnished 8α-acetoxy-4-epiguaioxide (9 mg) as colorless plates (sublimation, retention time 11·1 min), m.p. 106–107°, $[\alpha]_D^{21}$ —56·3° (± 1·3°) (*c* 0·734); IR (Nujol) 1725 and 1250 cm^{-1} (OAc); NMR 9·12 (3H, d, $J = 6$) and 9·05 (3H, d, $J = 6$) [Me on C-4 and C-10], 8·81 (3H, s) and 8067 (3H, s) [two Me on C-11], 7·94 (3H, s) [OAc] and 4·83 (1H, m) [H on C-8] (Found: C, 72·64; H, 10·10. $C_{17}H_{28}O_3$ requires C, 72·82; H, 10·06%).

Conversion of 8α-*hydroxyguaioxide* (IVa) *into guaioxide* (II). Mesyl chloride (0·1 ml) was added to a cooled soln of IVa (50 mg) in pyridine (1 ml) and the mixture was left overnight at room temp. Ice-water was added to the mixture, which was extracted with $CHCl_3$. The extract was washed, dried (Na_2SO_4) and evaporated leaving a crystalline substance (52 mg), recrystallized from light petroleum to give the mesylate of IVa as colorless plates, m.p. 127–128° (Found: C, 60·68; H, 8·87. $C_{16}H_{28}O_4S$ requires C, 60·74; H, 8·92%). A soln of the mesylate (45 mg) in pyridine (2 ml) was refluxed for 17 h and poured into ice-water and extracted with ether giving a product, which was chromatographed on alumina (activity IV, 10 g). Elution with light petroleum gave 8-dehydroguaioxide (VIII) as a colorless oil (17 mg), IR (film) 1645 cm^{-1} (—CH=CH—); NMR 9·02 (6H, d, $J = 6$) [Me on C-4 and C-10], 8·83 (3H, s) and 8·76 (3H, s) [two Me on C-11], and 4·57 (2H, m) [H on C-8 and C-9]. Successive elution with light petroleum-ether (4:1) recovered the mesylate (7 mg) unchanged (TLC and IR spectrum).

A mixture of VIII (15 mg) and Adams' catalyst (10 mg) in MeOH (5 ml) was hydrogenated at room temp to give guaioxide (II) as a colorless oil (13 mg) (IR spectrum and retention time).

Huang-Minlon reduction of 4α-*hydroxy*-8-*oxoguaioxide* (VI). A mixture of VI (87 mg), $NH_2NH_2 \cdot H_2O$ (80%, 1·2 ml), triethylene glycol (8 ml) and KOH (400 mg) was heated at 125–135° for 1 hr, the temp was gradually raised, and heating continued at 200–210° for 2 hr. Water was added to the mixture, which was extracted with ether. The extract was washed, dried (Na_2SO_4) and evaporated to leave a pale yellow oil (78 mg). This oil was purified by chromatography to give 4α-hydroxyguaioxide (V) as a colorless oil (52 mg) (IR spectrum and retention time).

LAH reduction of 4α-*hydroxy*-8-*oxoguaioxide* (VI). A soln of VI (82 mg) in dry ether (5 ml) was added portionwise to a suspension of LAH (45 mg) in dry ether (5 ml), and the mixture was stirred at room temp for 1 h. The reaction mixture was decomposed by addition of water and extracted with ether. The extract was washed, dried (Na_2SO_4) and evaporated to give a crystalline substance (77 mg), recrystallized from ether-light petroleum yielding 4α,8β-dihydroxyguaioxide (IXa) as colorless prisms, m.p. 132–134°, $[\alpha]_D^{20}$ —10·1° (± 0·7°) (*c* 0·725); IR ($CHCl_3$) 3600 and 3435 cm^{-1} (OH); NMR 9·07 (3H, d, $J = 6$) [Me on C-10], 8·78 (3H, s), 8·68 (3H, s) and 8·50 (3H, s) [Me on C-4 and C-11], and 6·20 (1H, m) [H on C-8] (Found: C, 70·95; H, 10·25. $C_{15}H_{26}O_3$ requires C, 70·83; H, 10·30%).

Acetylation of VI with Ac_2O and pyridine gave 8β-acetoxy-4α-hydroxyguaioxide (IXb) as colorless plates (from light petroleum), m.p. 83–85°, IR (CCl_4) 3600 (OH), 1735 and 1250 cm^{-1} (OAc) (Found: C, 69·29; H, 9·45. $C_{17}H_{28}O_4$ requires C, 68·89; H, 9·52%).

Conversion of 8β-*acetoxy*-4α-*hydroxyguaioxide* (IXb) *into torilolone acetate* (XIIb). Thionyl chloride (0·2 ml) was added to a cooled soln of IXb (210 mg) in pyridine (3 ml) and the mixture was left in an ice-bath for 10 min. Ice–water was added to the mixture, which was extracted with $CHCl_3$. The extract was washed, dried (Na_2SO_4) and evaporated leaving a residue (230 mg), which was chromatographed on alumina (activity V, 30 g). Elution with light petroleum-ether (98:2) yielded 8β-acetoxy-3-dehydroguaioxide (X) as colorless prisms (sublimation, 165 mg), m.p. 62–63°, IR (CCl_4) 1735, 1250 (OAc) and 1658 cm^{-1} (—CH=C<); NMR 9·04 (3H, d, $J = 6$) [Me on C-10], 8·77 (3H, s) and 8·53 (3H, s) [two Me on C-11], 8·33 (3H, d, $J = 1·2$) [Me on C-4], 7·97 (3H, s) [OAc], 5·10 (1H, broad) [H

on C-8], and 4·60 (1H,m) [H on C-3] (Found: C, 73·35; H, 9·32. $C_{17}H_{26}O_3$ requires C, 73·34; H, 9·41%).

A soln of diborane (50 mg) in dry THF (1·5 ml) was added dropwise to a cooled soln of X (110 mg) in dry THF (3 ml), and the mixture was left under N_2 at room temp for 30 min. Excess of diborane was decomposed by adding small pieces of ice, and the soln was concentrated. To this soln, Jones reagent (0·3 ml) was added and the mixture was stirred for 5 min. Working up in the usual manner gave a residue (134 mg), which was chromatographed on silica gel (15 g). Elution with light petroleum–ether (4:1) provided 8β-acetoxy-3-oxo-4-epiguaioxide (XI) as colorless prisms (64 mg, from light petroleum), m.p. 134–136°, $[\alpha]_D^{21}$ + 90·2° (± 2·2°) (c 0·600); IR (CCl_4) 1745 and 1245 cm^{-1} (cyclopentanone and OAc); NMR 8·99 (3H, d, J = 6) and 8·93 (3H, d, J = 7) [Me on C-4 and C-10], 8·79 (3H, s) and 8·50 (3H, s) [two Me on C-11], 7·95 (3H, s) [OAc], and 5·10 (1H, broad) [H on C-8]; ORD $[\phi]_{400}$ + 1413°, $[\phi]_{319}$ + 12954°, $[\phi]_{274}$ −13700°, $[\phi]_{238}$ −9028° (c 0·600, t=21°). (Found: C, 69·40; H, 9·04. $C_{17}H_{26}O_4$ requires C, 69·36; H, 8·90%).

A sample of XI (50 mg) was placed in a light petroleum soln on a column of alumina (activity V, 5 g) for 30 min. Elution with ether gave torilolone acetate (XIIb) as a viscous oil (49 mg), $[\alpha]_D^{24}$ −60·7° (± 0·8°) (c 1·246, MeOH); UV λ_{max} 240 mμ (ε 15400) (EtOH); IR (CCl_4) 3600, 3480 (OH), 1748, 1240, 1225 (OAc), 1705 and 1645 cm^{-1} (cyclopentenone); NMR 8·97 (3H, d, J = 5·5) [Me on C-10], 8·72 (6H, s) [two Me on C-11], 8·27 (3H, s) [Me on C-4], 7·91 (3H, s) [OAc], and 4.67 (1H, broad) [H on C-8] (Found: C, 69·17; H, 9·04. $C_{17}H_{26}O_4$ requires C, 69·36; H, 8·90%). This was identical with an authentic sample prepared by acetylation of torilolone (XIIa)[7] with Ac_2O and pyridine at room temp (IR and NMR spectra).

Conversion of 4α-*hydroxyguaioxide* (V) *into* 8-*deoxytorilolone* (XIV). Thionyl chloride (0·3 ml) was added to a cooled soln of V (305 mg) in pyridine (3 ml) and the mixture was left at room temp for 2 h. Working up in the usual manner gave a crude product (243 mg), which was chromatographed on alumina (activity IV, 30 g). The first elution with light petroleum furnished 3-dehydroguaioxide (67 mg) as an oil, IR (film) 1658 cm^{-1} (—CH=C<); NMR 9·10 (3H, diffused d) [Me on C-10], 8·78 (3H, s) and 8·67 (3H, s) [two Me on C-11], 8·33 (3H, d, J = 1·2) [Me on C-4], and 4·63 (1H, m) [H on C-3]. Successive elution with light petroleum gave a mixture (105 mg) of 3-and 4-dehydroguaioxides (NMR spectrum).

3-Dehydroguaioxide (38 mg) was treated with diborane followed by Jones reagent as described for X to give 3-oxo-4-epiguaioxide (XIII) as colorless prisms (sublimation, 39 mg), m.p. 47–49°, $[\alpha]_D^{23}$ +68·7° (± 1·1°) (c 1·002); IR ($CHCl_3$) 1740 cm^{-1} (cyclopentanone); NMR 9·03 (3H, d, J = 6) and 8·94 (3H, d, J = 6·5) [Me on C-4 and C-10], 8·80 (3H, s) and 8·64 (3H, s) [two Me on C-11]; ORD $[\phi]_{400}$ + 1226°, $[\phi]_{319}$ + 12680°, $[\phi]_{275}$ −14582°, $[\phi]_{250}$ −10564° (c 0·501, t=23°). (Found: C, 76·46; H, 10·08. $C_{15}H_{24}O_2$ requires C, 76·22; H, 10·24%).

A sample of XIII (39 mg) was placed in a light petroleum soln on a column of alumina (activity V, 5 g) for 30 min. Elution with ether gave 8-deoxytorilolone (XIV) as colorless prisms (sublimation, 36 mg), m.p. 70-71°, $[\alpha]_D^{21}$ −49·7° (± 1·1°) (c 0·794, MeOH); UV λ_{max} 241 mμ (ε 14900) (EtOH); IR ($CHCl_3$) 3580, 3400 (OH), 1686 and 1635 cm^{-1} (cyclopentenone); NMR 8·95 (3H, diffused d) [Me on C-10], 8·76 (6H, s) [two Me on C-11] and 8·31 (3H, s) [Me on C-4] (Found: C, 75·99; H, 10·23. $C_{15}H_{24}O_2$ requires C, 76·22; H, 10·24%).

Alkaline treatment of 8-*deoxytorilolone* (XIV). A sample of XIV (25 mg) was treated with 3% MeOH–KOH under reflux for 2h. The mixture was diluted and extracted with $CHCl_3$. The extract was washed, dried (Na_2SO_4) and evaporated to leave a residue (24 mg), which was chromatographed on alumina (activity V, 5 g). Elution with light petroleum–ether (4:1) recovered XIV unchanged (11 mg) (TLC and IR spectrum). Successive elution with light petroleum–ether (1:1) gave 8-deoxy-1-epitorilolone (XV) as viscous oil (12 mg), $[\alpha]_D^{22}$ + 185·4° (±3·9°) (c 0·569, MeOH), IR ($CHCl_3$) 3560 (OH), 1685 and 1630 cm^{-1} (cyclopentenone); NMR 9·36 (3H, d, J = 6·5) [Me on C-10], 8·77 (3H, s) and 8·73 (3H, s) [two Me on C-11], and 8·32 (3H, s) [Me on C-4] (Found: C,76·10; H, 10·14. $C_{15}H_{24}O_2$ requires C, 76·22; H, 10·24%).

Conversion of 8-*deoxytorilolone* (XIV) *into* 1β,5β-*dihydroguaiol* (XVII). A mixture of XIV (100 mg) and 10% Pd-C (50 mg) in EtOH (8 ml) was hydrogenated at room temp. When 1·1 mol H_2 had been absorbed, the reaction ceased, and the mixture was worked up in the usual way to give a residue (105 mg). The residue showed two peaks of retention times 17·7 min (48%) and 20·8 min (52%) on GLC (5% diethyleneglycol succinate; 200°; He 200 ml/min).

A soln of the mixture (XVI, 62 mg) thus obtained and $NH_2NH_2 \cdot H_2O$ (80%, 1·2 ml) in triethyleneglycol (8 ml) was heated with KOH (400 mg) at 140° for 30 min, the temp was gradually raised, and heating continued at 190–195° for 2 h. The reaction mixture was diluted with water and extracted with ether. The extract was washed, dried (Na_2SO_4) and evaporated to give a product (58 mg), which showed two peaks of retention times 15·7 min (53%) and 17·6 min (47%) on GLC (5% phenyl-diethanolamine succinate; 160°; He 100 ml/min). The compound of retention time 15·7 min was 1β,5α-dihydro-4ξ-guaiol. m.p. 65–67°, $[\alpha]_D^{25}$ −20·5° (± 0·5°) (*c* 1·165). The compound of retention time 17·6 min was 1β,5β-dihydroguaiol (XVII), colorless prisms (sublimation), m.p. 26°, $[\alpha]_D^{22}$ + 65·7° (± 2·0°) (*c* 0·519); NMR 9·15 (3H, diffused d) and 9·00 (3h, diffused d) [Me on C-4 and C-10], and 8·84 (6H, s) [two Me on C-11] (Found: C, 80·15; H, 12·54. $C_{15}H_{28}O$ requires C, 80·29; H, 12·58%) which was identical with an authentic sample prepared by hydrogenation of guaiol (IR spectrum and retention time).

Hydrogenation of guaiol (I). A soln of I (4 g) in EtOH (25 ml) was hydrogenated over Raney Ni (2 g) at 100°/120 atm for 14 h. The product (4.03 g), after filtration of the catalyst and removal of EtOH, showed two peaks of retention times 17·6 min (32%) and 20·3 min (68%) on GLC (5% phenyldiethanolamine succinate; 160°; He 100 ml/min). The compound of retention time 17·6 min was 1β,5β-dihydroguaiol (XVII), m.p. 26° (sublimation). The compound of retention time 20·3 min was 1α,5α-dihydroguaiol, m.p. 77–79°, $[\alpha]_D^{22}$ −55·4° (± 1·9°) (*c* 0·493).

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