Structure Elucidation of Guaioxide by Microbial Oxidation

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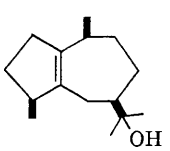
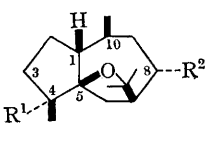
GUAIOXIDE is a sesquiterpene constituent of guaiac wood oil.[1] It is also obtained by acid-catalysed cyclization[1–3] of guaiol (I), the absolute configuration of which is known,[4] and four possible structures have been suggested.[1–3] In connection with the structure elucidation of liguloxide[5] we have studied the structure of guaioxide, and have suggested the stereochemistry (II).

In order to understand better the opening of the ditertiary ether linkage in guaioxide, we examined its microbial oxidation. From thirty kinds of micro-organisms we selected *Mucor parasiticus* BAIN (ATCC 6476) as it oxidized guaioxide to produce three hydroxylated compounds.

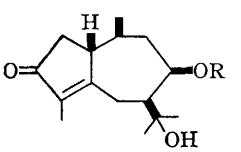
One of the products (IIIa), $C_{15}H_{26}O_3$,† m.p. 172—173°, $[\alpha]_D$ − 34·1°, was identified as 4α,8α-dihydroxyguaioxide by correlation with torilolone, of known structure (VIa).[6] Chromium trioxide



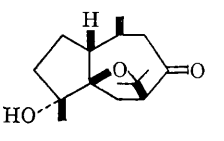
(I)



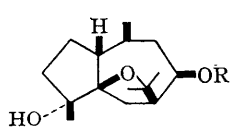
(II) $R^1 = R^2 = H$
(IIIa) $R^1 = R^2 = OH$
(IIIb) $R^1 = OH$, $R^2 = OAc$
(IVa) $R^1 = H$, $R^2 = OH$
(IVb) $R^1 = H$, $R^2 = OAc$
(V) $R^1 = OH$, $R^2 = H$



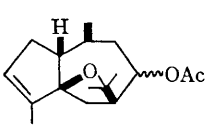
(VIa) R = H
(VIb) R = Ac



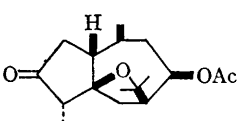
(VII)



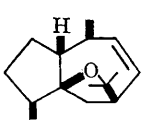
(VIIIa) R = H
(VIIIb) R = Ac



(IX) 8β-OAc
(XI) 8α-OAc



(X)



(XII)

CHART 1

† All new compounds gave satisfactory elemental analyses.

oxidation of compound (IIIa) gave a hydroxy-ketone (VII), m.p. 72—74°, $[\alpha]_D$ + 93·6°, ν_{max} 3600 and 1688 cm.$^{-1}$, which was reduced with lithium aluminium hydride to give an epimeric diol (VIIIa), m.p. 132—134°, $[\alpha]_D$ − 10·1°. Dehydration of its acetate (VIIIb) with thionyl chloride and pyridine yielded an unsaturated acetate (IX), m.p. 62—63°, which contains a –CH=CMe– grouping [n.m.r. τ 8·33 (3H) and 4·60 (1H)]. Hydroboration followed by oxidation with Jones reagent converted compound (IX) into a keto-acetate (X), m.p. 134—136°, $[\alpha]_D$ + 90·2°, ν_{max} 1745 cm.$^{-1}$ (five-membered ring ketone and OAc), the i.r. absorption due to the keto-group confirmed that the tertiary hydroxyl group in (IIIa) was located on C-4 and not C-10. Chromatography of the keto-acetate (X) on alumina gave rise to torilolone acetate (VIb), a viscous oil, $[\alpha]_D$ − 60·7°, its structure being confirmed by comparison with an authentic sample prepared by acetylation of torilolone.[6] This fact showed that the secondary hydroxyl group in (IIIa) is present in the 8α-position, since the hydroxyl group had been epimerized during a series of reactions. The ether bridge in guaioxide is clearly β-oriented, and thus the absence of intramolecular hydrogen bonding in (IIIa) (i.r. spectrum) shows that the configuration of the hydroxyl group on C-4 is α, and the product (IIIa) is 4α,8α-dihydroxyguaioxide.

The correlation of compound (IIIa) with torilolone further corroborated that the ethereal oxygen in guaioxide is linked to C-5 and not C-1, providing that the microbial oxidation does not alter the relative arrangement of atoms in guaioxide. That this assumption was correct was confirmed in the course of structure elucidation of another two microbial oxidation products (IVa and V).

Product (IIIa) was converted into a second product (IVa), $C_{15}H_{26}O_2$, m.p. 82—83°, $[\alpha]_D$ −23·0°, which proved to be 8α-hydroxyguaioxide: the acetate (IIIb), m.p. 69—71°, was dehydrated to give the 3-dehydro-compound (XI). This was hydrogenated to form a 1 : 1 mixture of 8α-acetoxyguaioxide and its C-4 epimer; the former was identical with the acetate (IVb), m.p. 56—57°, of product (IVa) (mixed m.p. and i.r. spectrum). Dehydration of 8α-hydroxyguaioxide (IVa) with methanesulphonyl chloride and pyridine yielded the 8-dehydro-compound (XII) (n.m.r., –CH=CH– grouping), hydrogenation of which resulted in the regeneration of guaioxide (II).

The last product (V), $C_{15}H_{26}O_2$, an oil, $[\alpha]_D$ −35·4°, was also prepared by Huang-Minlon reduction of the hydroxy-ketone (VII), thus establishing it to be 4α-hydroxyguaioxide.

The correlations among the compounds (II, III, IV, and V) show that all three microbial oxidation products contain the same carbon–oxygen skeleton as guaioxide itself. 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 through a simple replacement of the hydrogen in the position to be hydroxylated.[7]

The remaining problem is the stereochemistry at C-1 in guaioxide. The o.r.d. determination of the keto-acetate (X) showed a strong positive Cotton effect curve (a = + 267). Inspection of the molecular model of the compound and application of the octant rule[8] indicate that the configuration at C-1 in (X) is β, and from the very large amplitude the methyl group at C-4 is assumed to have α-orientation. Since it is obvious that inversion at C-1 cannot occur in the course of transformations from guaioxide to the keto-acetate (X), guaioxide should be represented by the formula (II).

Chemical proof for the β-configuration of C-1 in guaioxide was obtained by the conversion of a ketone (XIII) into the known 1β,5β-dihydroguaiol (XVII).[9,10] The ketone (XIII) [m.p. 47—49°, o.r.d. positive Cotton effect (a = +273)] was prepared from 4α-hydroxyguaioxide (V) by a similar sequence of reactions as in the dihydroxyguaioxide (III) series. Chromatography of the ketone on alumina gave deoxytorilolone (XIV), m.p. 70—71°, $[\alpha]_D$ − 49·7°, which on heating with 3% methanolic potassium hydroxide yielded a 1 : 1 equilibrium mixture ($[\alpha]_D$ + 66·1°) of compound (XIV) and its C-1 epimer (XV), an oil, $[\alpha]_D$ + 185·4°. Since both the compounds were shown to be stable on alumina chromatography, and compound (XIV) was obtained as a sole product from the ketone (XIII), the configuration at C-1 of compound (XIV) must be the same as that of the ketone (XIII), and thus that of guaioxide.

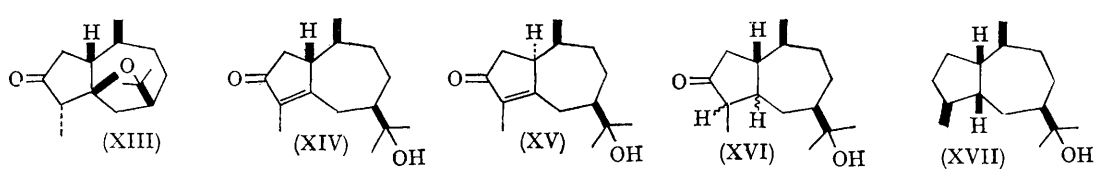


CHART 2

Deoxytorilolone (XIV) was hydrogenated to furnish a 1 : 1 mixture of two dihydro-compounds (XVI), isomers of *cis*- and *trans*-ring junctions. Huang-Minlon reduction of the mixture afforded a mixture of deoxo-derivatives (1 : 1), which were separated by gas chromatography. One of these was found to be identical with an authentic sample of 1β,5β-dihydroguaiol (XVII), m.p. 26°, $[\alpha]_D$ +65·7°, prepared by high-pressure hydrogenation[11] of guaiol (I) followed by gas chromatographic separation (m.p., i.r. spectrum, and retention time). This was proof of the β-configuration of C-1 in guaioxide, and also the first example of the preparation of pure 1β,5β-dihydroguaiol.[3,9,12]

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