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STUDIES IN THE BIOCHEMISTRY OF MICROORGANISMS

Part XIX.—Structure and Stereochemistry of Kamusol, a Metabolic Product of Aspergillus sulphureus

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The structure and stereochemistry of *Kamusol* $C_7H_{14}O_7$, m.p. 158-159°C, $[\alpha]_D^{25}$—9.09, a metabolite of *Aspergillus sulphureus* has been established as I.

Aspergillus sulphureus (Thom and Church) has been isolated from the soil of different parts of Pakistan. The isolation and structures of five metabolites, alongwith the isolation of kamusol, $C_7H_{14}O_7$, m.p. 158–159°C, have been described earlier from the above mold when grown on synthetic medium, enriched with carrot extract.[1] In the present paper the structure and stereochemistry of kamusol, $C_7H_{14}O_7$, have been described.

The mold was grown on Czapek–Dox medium enriched with carrot extract. After three weeks the broth was separated from the mycelium and the mycelium was dried, powdered and extracted with petroleum ether, ethyl acetate and finally with methanol, for 48 hr in each case. On evaporation of the methanol a reddish brown oil was obtained, which was taken up in water and then methanol was added to it. Crystalline material was obtained, which was recrystallized from methanol, colourless rectangular plates of kamusol, m.p. 158–159°C were obtained.

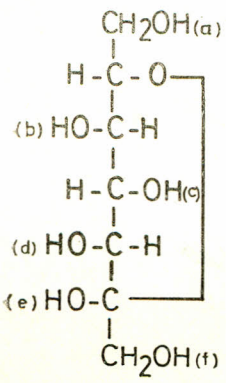
The UV spectrum in water showed absorption bands at λ_{max} 260 nm (ε 30). Its IR spectrum showed very strong absorption bands in the region 3140~3320 cm^{-1} for hydroxyl groups. Total absence of any band in the carbonyl region and the region above 1500 cm^{-1} was clearly indicative of simple aliphatic type of compound.

The PMR spectrum in DMSO showed a sharp singlet at τ 6.55 (4*H*) accounting for the presence of the two methylene groups. Another sharp singlet appeared at τ 6.35 (4*H*) indicating the presence of four methine protons in the molecule. The six hydroxyl protons appeared between τ 5.76 ~ τ 5.45. The protons of hydroxyl groups (a and f) showed singlet at τ 5.45 (2*H*). The protons of hydroxyl group (e and d) appeared as doublet, centred at τ 5.54 (2*H*; *J* 2 Hz). The remaining two protons of two hydroxyl groups (b and c) appeared as doublet, centred at τ 5.74 (2*H J* 10 Hz), thus accounting for all the protons present in the molecule. All the above data gave structure (I) for kamusol.

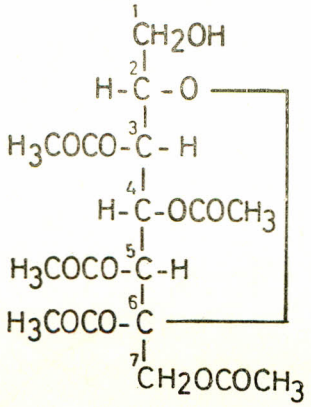
This structure was further supported by its mass fragmentation pattern. The mass spectrum showed a very weak molecular ion peak at m/e 210^+. The first intense peak at m/e 193^+ (M^+–17; OH). This ion underwent fragmentation with the loss of 30 mass units (CH_2O) to give rise to a peak at m/e 163^+, which again lost 30 mass units (CH_2O) to give the base peak at m/e 133^+. From here onwards there were three successive losses each of 30 mass units ($-CH_2O$) to give rise to peaks at m/e 103^+, 73^+, 43^+ respectively. The other significant peaks appeared at 192^+, 179^+, 161^+ and 61^+ corresponding to the molecular ion species as shown in Chart 1. Most of these ionic species were formed by the migration and rearrangement of one proton to the adjacent carbon.

The structure assigned to kamusol as (I) was further confirmed from the PMR and mass spectral studies of its pentaacetyl derivative: (II) $C_{17}H_{24}O_{12}$, prepared by acetylation with acetic anhydride in pyridine and obtained as colourless plates, m.p. 107–108°C $[\alpha]_D^{25}$–26.5. Its UV showed a shoulder at λ_{max} 260 nm (ε131). Its IR showed a sharp absorption band at 3410 cm^{-1} for OH group. A sharp band appeared at 1740 cm^{-1} due to carbonyl of $OCOCH_3$ group in the molecule.

The PMR spectrum showed three large singlets at τ 7.9 (3*H*), τ 7.95 (6*H*) indicating the presence of five $OCOCH_3$ groups in the molecule. One triplet was centred at τ 5.8 (1*H*) due to methine proton absorption in the environment of —CH_2—C*H*—O group and one doublet appeared at τ 4.6 (2*H*) for one methylene group (O—CH_2—CH—O). A multiplet centred at τ 4.8 (1*H*) for a methine proton, showing long range coupling with a methylene and methine groups in the molecule. There was a broad hump at τ 4.42 (1*H*) for the proton of the hydroxyl group, which disappeared on deuteration, thus confirming the presence of remaining one hydroxyl group in the molecule, which was not easily acetylated under above conditions. This hydroxyl group will be on the carbon atom 1, adjacent to the carbon atom containing ether linkage.



I



II

This structure was further supported by its mass fragmentation pattern which did not show a molecular ion peak at m/e 420^+. The first most intense peak appeared at m/e 361^+, formed by the loss of 59 mass units ($—OCOCH_3$), which is a characteristic behaviour of acetyl derivatives of sugars, corresponding to a sugar consisting of more than five–membered ring. This ion underwent fragmentation to lose 72 ($C_3H_4O_2$) and 30 mass units (CH_2O) sucessively to form peaks at m/e 289^+ and 259^+ indirespectively. The loss (CH_2O) was a clear cation of an unacetylated CH_2OH group present in the molecule. This loss occurred due to the migration of one proton to the adjacent carbon. This ion then underwent three successive losses of 42 mass units for $CO.CH_2$ group giving rise to very intense peaks at m/e 217^+, 175^+ and 133^+. The ion at m/e 133^+ becomes a hydroxy sugar and the fragmentation is parallel to that of kamusol.

All the above losses occurred with the migration of one proton to its adjacent atom. The other most significant peaks were at m/e 103^+, and 85^+. All these peaks are outlined in Chart 2.

The above structure was finally confirmed by its chemical degradation. Kamusol was oxidized with sodium metaperiodate and the consumption of periodate was followed by its UV absorption band at λ_{max} 222 nm. After 24 hr 3 moles of periodate were consumed, which clearly indicated the presence of six hydroxyl groups in the molecule.

Attempted hydrogenation of kamusol in acetic acid in presence of 5% Pd–C catalyst yielded an unchanged product, indicating the absence of unsaturation in the molecule.

Stereochemistry.

The relative stereochemistry of kamusol can be determined on the basis of coupling constants of different proton in the NMR spectra.

The OH protons (a and f) showed a sharp singlet at τ 5.45 (2*H*) due to identical environments. The OH protons (d and e) appeared as a doublet centred at τ 5.54 (2*H*; *J* 2 Hz) indicating *cis*-configuration for these groups. The protons of the hydroxyl groups (b and c) showed a fine doublet, centred at τ 5.74 (2*H*; *J* 10 Hz) indicating the *trans*-configuration of these groups. For its absolute configuration ORD studies are being initiated and the findings will be communicated later.

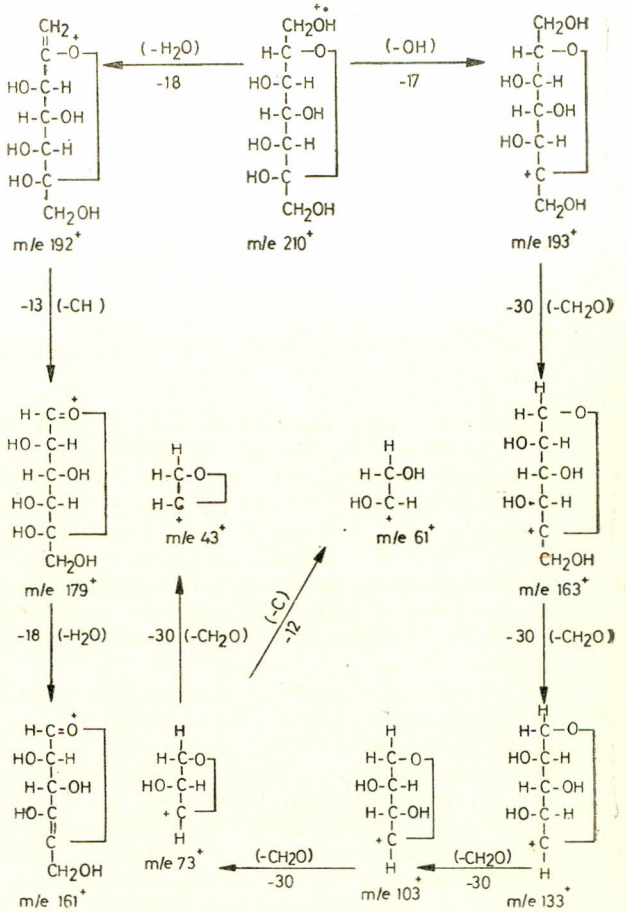


Chart 1.—Mass fragmentation pattern of kamusol.

Experimental

M. ps were taken on Kofler block and are uncorrected UV spectra were taken in water on a Beckman DB spectrophotometer. IR spectra were determined with a Perkin-Elmer 237-E instrument in KBr, unless otherwise stated. PMR spectra were recorded on DP 60 Varian Associate NMR machine, using TMS as an internal standard. Mass spectra were measured on an AE-I MS-9 at 70 eV. Petroleum ether used had b.p. 65–85°C.

Isolation of Kamusol.—Kamusol was isolated from *Aspergillus sulphureus* grown on Czapek–Dox

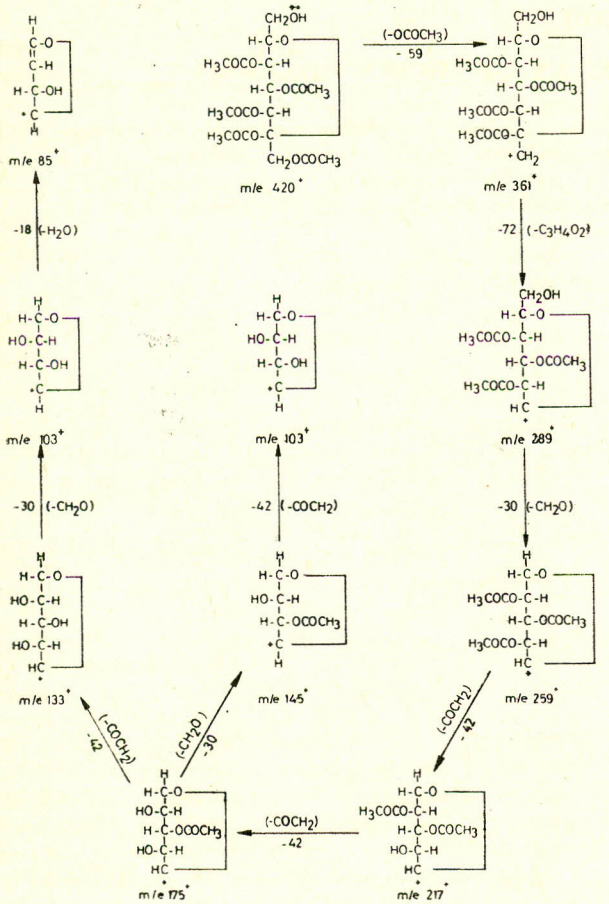


Chart 2.—Mass fragmentation pattern of penta-acetyl kamusol.

medium enriched with carrot extract as reported earlier.[1] It was isolated from the mycelium, dried at 60°C in an oven for 48 hr. The mycelium was extracted successively from petroleum ether, ethyl acetate and then methanol. The methanol extract yielded pure kamusol as colourless rectangular plates; m.p. 158–159°C, $[\alpha]_D^{25}$ –9.09.

Acetylation of Kamusol.—Kamusol (200 mg) was dissolved in a mixture of anhydrous pyridine (5 ml) and acetic anhydride (10 ml), and the reaction mixture was refluxed on a water-bath (½ hr) and left overnight. The reaction mixture was poured over crushed ice and extracted with ethyl acetate three or four times and the combined ethyl acetate extract was washed with 2N HCl to remove pyridine. The ethyl acetate extract was dried (Na_2SO_4), filtered and the solvent removed, yielding a crystalline product which was recrystallized from methanol. Colourless plates of pentaacetyl kamusol, m.p. 107–108°C, (235 mg). (Found: C, 48.80; H, 6.08; O, 45.12%; $C_{17}H_{24}O_{12}$ requires: C, 48.57; H, 5.76; O, 45.68%.)

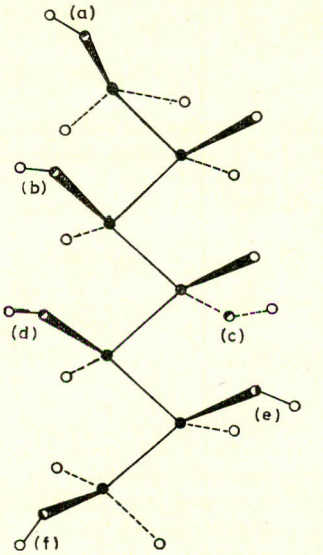


Fig. 1. — Kamusol; ● = carbon ○ = hydrogen, ◔ = oxygen.

Periodate Oxidation of Kamusol.—Kamusol (42 mg, 0.0002 mole) was treated with sodium metaperiodate (170.4 mg, 8 moles) in 250 ml water. The consumption of periodate was followed through UV absorption at λ_{max} 222 nm.[2] After 24 hr three moles out of the eight moles of sodium metaperiodate were consumed.

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References

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2. R.D. Guthrie, *Methods in Carbohydrate Chemistry* (Academic Press, New York, 1962), Vol. 2, p. 432.