Pakistan J. Sci. Ind. Res., Vol. 13, No. 4, December 1970

STUDIES IN THE BIOCHEMISTRY OF MICROORGANISMS

Part XV.—Synthesis of Amudol—a Mold Metabolite of *Penicillium martinsii* Biourge

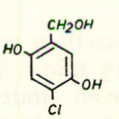
A. Kamal, Nilofer Kazi, Shaheen A. Husain and Asaf A. Qureshi

P.C.S.I.R. Laboratories, Karachi 39

(Received September 15, 1970)

The synthesis of amudol (2,5-dihydroxy-4-chlorobenzyl alcohol), a metabolite of *Penicillium martinsii* Biourge, has been carried out starting from 2,5-dihydroxytoluene. A novel way has been employed to isolate the synthetic amudol as 2,5-dihydroxy-4-chloro-3,6-ditritiated benzyl alcohol.

In the earlier communications[1-4] we have described the isolation, characterization and biosynthesis of amudol (I) which is a very interesting molecule due to the oxidation of the starting unit into benzyl alcohol, chlorination at position 4 in the benzene ring and the occurrence of the phenomenon of an "NIH" shift[5] in this molecule. The presence of all these interesting features in such a simple molecule, initiated us to synthesize the early possible precursors, *in vitro*, which led ultimately to its full synthesis. In the present paper the synthesis of amudol has been described, starting from a known compound: 2,5-dihydroxytoluene.



(I)

2,5-Dihydroxytoluene was acetylated with pyridine and acetic anhydride, yielding pure 2,5-diacetoxytoluene. The IR spectrum (thin film) showed absence of any hydroxyl group, but showed a strong absorption band at 1750 cm^{-1} for acetyl group. It is interesting to observe that the benzenoid stretching decreased to 8%. The confirmation of the formation of diacetoxy derivative was further supported by its UV spectrum, which showed a λ_{max} at 266 mμ (ε 653.3) and 257 mμ (ε 618). The blue or red shift was not observed with a drop of base.

2,5-Diacetoxytoluene was chlorinated under controlled conditions. The resulting mixture of chlorinated compounds contained the desired 2,5-diacetoxy-4-chlorotoluene. Due to scarcity of the material the chlorinated compounds could not be separated at this stage. Their IR spectra showed strong absorption bands at 1750 cm^{-1} ($-OCOCH_3$) 1600 cm^{-1} and 1580 cm^{-1} for benzenoid stretching. The chlorine absorption band appeared at 1000 cm^{-1}. The UV spectrum* showed λ_{max} at 265 mμ (ε 589.6) which was consistent with the known chloro-substituted benzenoid compounds, having acetyl groups as the other substituents on the benzene ring. This low absorption might be due to the electronegative chlorine.

The above mixture of chlorinated diacetoxy derivatives was oxidised with $KMnO_4$ in acetone which gave a very good yield of the chlorinated diacetoxybenzoic acids. Their IR spectrum (in chloroform) showed absorption bands between 3500 3100 cm^{-1}, 2900 2600 cm^{-1} and then at 1700 cm^{-1} for carboxylic group. Apart from these bands there was a strong absorption band at 1750 cm^{-1} and 1740 cm^{-1} for the acetyl and the ester groups respectively. The UV. Spectrum showed a strong absorption band at λ_{max} 268 mμ (ε 988.1). The somewhat low absorption value of the ester group was consistent with the already known example of methyl benzoate. The esters were reduced to benzyl alcohol with lithium aluminium hydride, yielding a mixture of three compounds, one of which was diacetoxyamudol. The quantity of the material so obtained was so small (15 mg) that isolation through preparative thin-layer chromatography was not attempted. Instead separation through radio-dilution method was tried. The cold mixture containing 2, 5-diacetoxy-4-chlorobenzyl alcohol was dissolved in triethylamine, containing tritium oxide and heated (100°C) in a sealed tube for 2 hr. The tritiated mixture of products containing diacetoxyamudol was hydrolysed with alcoholic potassium hydroxide under nitrogen. To the worked up reaction product cold amudol (70 mg) was added. Since amudol is practically insoluble in benzene and since its dichloro and trichloro derivatives are soluble, the mixture of 'hot' and 'cold' *amudol* with the other chlorinated products were dissolved in

*All ε values are calculated on the assumption that only monochloro derivatives are formed.

chloroform and after removal of this solvent repeatedly extracted with benzene. The insoluble residue which was practically pure tritiated amudol (II) was crystallised from ether–benzene. The sequence of reactions is shown in Chart 1.

Experimental

Melting points were taken on a Kofler block and are uncorrected. IR spectra were taken on Perkin-Elmer-137 spectrophotometer. UV spectra were measured in methanol on a Beckman DK-2 spectrophotometer. Radioactivity is described in relative molar activity (r.m.a.). All the samples were crystallized and purified to constant radioactivity. Petroleum ether used had b.p. 65–85°C. Merck's silica gel PF 254 was used for TLC.

Acetylation of 2,5-Dihydroxytoluene.—2,5-Dihydroxytoluene (55 mg) was taken up in a mixture of anhydrous pyridine (1 ml) and acetic anhydride (1 ml), refluxed on a water-bath (1 hr) and left overnight. The reaction mixture was poured onto crushed ice, extracted with ethyl acetate and the extract washed repeatedly with 2N HCl, and then with water. The extract was dried (Na_2SO_4) and the solvent removed under reduced pressure to obtain almost pure 2,5-diacetoxytoluene as a light brown oil (51 mg, 55.4%). It gave no IR bands for OH.

Chlorination of 2,5-Diacetoxytoluene.—2,5-Diacetoxytoluene (41.6 mg, 0.0002 mole) was dissolved in chloroform (15 ml). Chlorine was passed through the cold solution very slowly (1 bubble/sec) for 3 min and the reaction mixture was irradiated with a 1000 W bulb (4 hr). Removal of solvent under reduced pressure gave a crystalline material which as shown by TLC could be various chlorine-substituted 2,5-diacetoxytoluenes including the desired 4-chloro-2, 5-diacetoxytoluene.

Oxidation of the Mixed Chloro 2,5-Diacetoxytoluenes.—The above mixture of chlorinated 2,5-diacetoxytoluenes (50 mg) was dissolved in anhydrous pure acetone (25 ml), and heated on water-bath. Powdered $KMnO_4$ (350 mg) was added in small portions after every 15 min for 2 hr and then left to stand overnight. The solvent was removed, the residue treated with 2N HCl and the reaction mixture was extracted with ethyl acetate (4 times). The combined extract was dried (Na_2SO_4) and the solvent removed under reduced pressure, yielding a mixture of chlorinated 2,5-diacetoxybenzoic acids (35 mg).

Esterification of Chlorinated 2,5-Diacetoxybenzoic Acids.—The mixture of the above chlorinated 2,5-diacetoxybenzoic acids (30 mg) was dissolved in ether (5 ml) containing methanol (1 ml), the

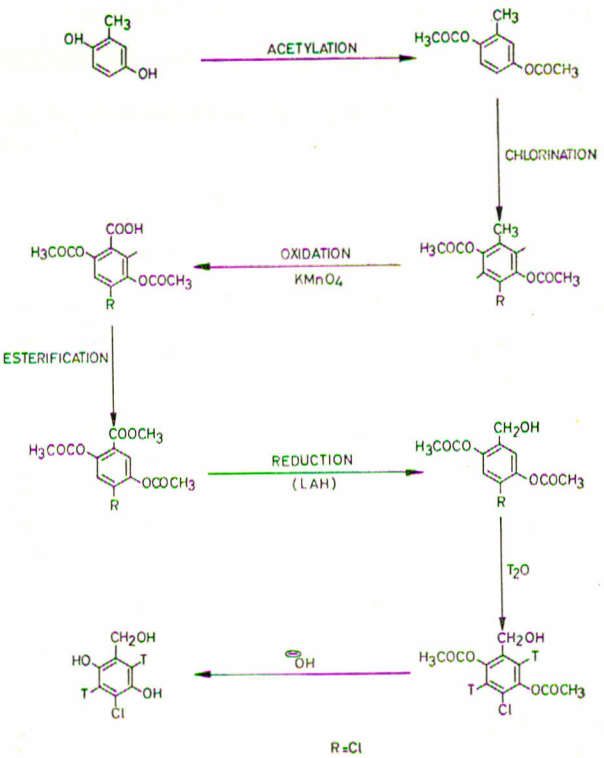


Chart 1.—Synthesis of tritiated amudol.

reaction mixture was then treated with excess of diazomethane for 15 min. The excess of diazomethane was removed under reduced pressure, yielding the various chloro-substituted methyl 2,5-diacetoxybenzoates (28 mg) as semisolid residue.

Reduction of Methyl Chloro-2,5-Diacetoxybenzoates.—The above methyl chloro-2,5-diacetoxybenzoates (25 mg) were dissolved in anhydrous ether, and while refluxing, small quantities of lithium aluminium hydride (200 mg) were added after every 10 min for 1 hr. The solvent was removed and the complex decomposed with 2N HCl, and thoroughly extracted with ethyl acetate. The ethyl acetate extract was dried (Na_2SO_4), filtered, and the solvent removed under reduced pressure, yielding chlorinated 2,5-diacetoxybenzyl alcohols (19 mg).

Labeling of Chloro 2,5-Diacetoxybenzyl Alcohols.—The above chloro 2,5-diacetoxybenzyl alcohols (15 mg) were dissolved in triethylamine (2 ml) and two drops of tritium oxide (specific activity 5 m.c.) were added to it. The tube was sealed in vacuum and heated in water-bath (2 hr). To the contents of the tube ethanol (5 ml) was added and the solvents removed under reduced pressure.

The residue was taken up in ethyl acetate, washed with a few drops of 2N HCl and water. Drying (Na_2SO_4) and removal of the solvent from the extract gave a mixture of chloro-substituted and partially hydrolysed 2,5-diacetoxybenzyl alcohols (15 mg).

Hydrolysis of Tritiated Mixture of Chlorinated 2,5-Diacetoxybenzyl Alcohol.—The above crude mixture (15 mg) was refluxed (½ hr) under N_2 with alcoholic KOH solution (10%, 5 ml). The solvent was removed, acidified with dil HCl and extracted with ethyl acetate three times. The combined extract was dried (Na_2SO_4), filtered, and the solvent removed from the filtrate to obtain chloro-dihydroxy-tritiated-benzyl alcohols (10 mg).

Isolation of Synthetic Amudol.—To the above mixture cold natural amudol (70 mg) was added, and the mixture was dissolved in chloroform. After removal of the solvent, the residue was repeatedly extracted with benzene, which removed all the other chlorinated products except amudol which remained undissolved and was crystallized from ether–benzene mixture, m.p. 146–147°C, r.m.a. 258108.

Acknowledgement.—Our thanks are due to Dr. M.L. Smith, Cento Scientific Secretary, Cento Scientific Coordination Board, Tehran, for the gift of tritium oxide, and Dr. Amir Muhamed, Director, Radiation Genetics Institute, Pakistan Atomic Energy Commission, for the loan of their radiocounter.

References

1. I.H. Qureshi, A. Kamal, R. Noorani, S. Aziz, and S.A. Hussain, Pakistan J. Sci. Ind. Res., **11,** 364 (1968).
2. A. Kamal, C.H. Jarboe, I.H. Qureshi, S.A. Hussain, N. Murtaza, R. Noorani and A.A. Qureshi, Pakistan J. Sci. Ind. Res., **13,** 236 (1970).
3. A. Kamal, S.A. Husain, N. Murtaza, R. Noorani, I.H. Qureshi and A.A. Qureshi, Pakistan J. Sci. Ind. Res., **13,** 240 (1970).
4. A. Kamal, S.A. Husain, N. Murtaza, A.A. Qureshi and M.A. Wahid, Pakistan J. Sci. Ind. Res., **13,** 378 (1970).
5. G. Guroff, J.W. Dalay, D.M. Ienna, J. Renson, B. Witkep and S. Undenfriend, Science, **117,** 1524 (1967).