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STUDIES IN THE BIOCHEMISTRY OF MICROORGANISMS

Part XVII.—Synthesis of Amudol—a Metabolic Product of Penicillium martinsii Biourge

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The synthesis of amudol (XI), a mold metabolite, has been carried out by two different routes starting from 2,5-dihydroxyacetophenone and 2,5-dihydroxybenzoic acid.

In the previous communication we have described the synthesis of amudol[1] (2,5-dihydroxy-4-chlorobenzyl alcohol) (XI), a metabolic product of *Penicillium martinsii* Biourge, starting from 2,5-dihydroxytoluene.[2] Since 2,5-dihydroxytoluene was available in a very small quantity, amudol, obtained towards the end of the synthesis was very small. Nevertheless, in order to separate amudol we employed radiodilution technique by tritiating amudol to identify its presence in the mixture. However, for reasons of low yield the synthesis could not be evaluated. In this communication we are reporting the synthesis of amudol, with 2,5-dihydroxyacetophenone (I) and 2,5-dihydroxybenzoic acid (VII) as starting materials as shown in Chart 1.

2,5-Dihydroxyacetophenone[3] (I) (m.p. 201°C) was prepared from hydroquinone diacetate. Its UV spectrum showed λ_{max} at 252 nm (ε 1575). With a drop of base a bathochromic shift was observed, giving a shoulder at 260 nm (ε 1393) and λ_{max} at 235 nm (ε 3799).

The IR spectrum showed absorption bands at 3240 cm^{-1} (free OH), 3050 cm^{-1} (hydrogen bonded OH), 1630 cm^{-1} ($COCH_3$), 1600 and 1560 cm^{-1} (benzene ring stretching).

The PMR spectrum in pyridine-d_6 showed a short singlet at τ 7.72 (3H,—COCH_3). In the benzenoid region there was a doublet at τ 3.5 (1H; *J* 10 c/s) and superimposed quartet centred at τ 3.27 (1H, *J* 10 and 2 c/s), indicating the *ortho* and *meta* environments of these two protons respectively. The third benzenoid proton appeared as an ill-defined doublet at τ 3.02 (1H, *J* 2 c/s) indicating a *meta* substitution on the benzene ring. The remaining two hydroxyl protons appeared at τ 1.75.

The above 2,5-dihydroxyacetophenone was acetylated with acetc anhydride in pyridine

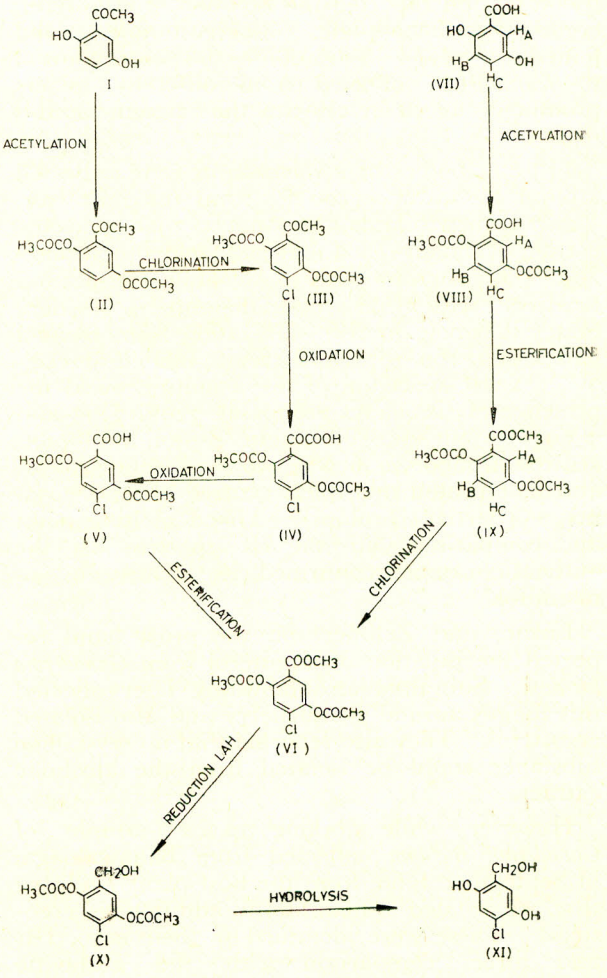

Chart 1

yielding 2,5-diacetoxyacetophenone (II) m.p. 68°C. Its UV spectrum showed λ_{max} at 282 nm (ε 2149) and 230 nm (ε 10980).

The IR spectrum supports the formation of its acetyl derivative which showed the absence of a hydroxyl group in the region 4000~3000 cm^{-1} but showed the presence of two sharp bands at 1750 cm^{-1} and at 1740 cm^{-1} for the carbonyls of acetoxyl groups. The carbonyl of the —$COCH_3$ group now appeared at 1680 cm^{-1} due to the removal of hydrogen bonding with the hydroxyl groups. The benzenoid stretching bands appeared at their usual place at 1602 and 1570 cm^{-1}.

The PMR spectrum of 2,5-diacetoxyacetophenone in $CDCl_3$ showed a sharp singlet at τ 7.92 (3H; $COCH_3$), two sharp singlets appeared at τ 7.88 and τ 7.78 for (2×3H, $OCOCH_3$) respectively. The lower chemical shift for one acetoxyl group was indicative of an electronegative group like —$COCH_3$ present on an adjacent position. The remaining three benzenoid protons were centred at τ 3.27 and τ 3.82 as multiplets.

The above 2,5-diacetoxy-acetophenone in chloroform, containing chlorine, on irradiation with a 1000 W bulb, yielded 2,5-diacetoxy-4-chloroacetophenone (III) which crystallised out from the solution almost pure (m.p. 54°C). Its UV spectrum showed λ_{max} 285 nm (ε 5692) and 230 nm (ε 15270).

The IR spectrum showed clear and sharp absorption bands at 1760 cm^{-1} for carbonyl of the acetoxy groups and 1685 cm^{-1} for carbonyl of —$COCH_3$ groups. Two small bands appeared at 1605 and 1565 cm^{-1} for benzenoid stretching. The chlorine absorption band appeared at 960 cm^{-1}.

The PMR spectrum of this compound in $CDCl_3$ showed three sharp singlets at τ 7.82, τ 7.76 and τ 7.6 (9H) for one —$COCH_3$ group and two —$OCOCH_3$ groups respectively. One —$OCOCH_3$ group appeared at τ 7.6 due to the presence of an electronegative group like chlorine adjacent to it. In the benzenoid region there were two sharp singlets appearing at τ 3.65 and τ 3.3 for two benzenoid protons *para* to each other. The remaining mother liquor obtained on removal of 2,5-diacetoxy-4-chloroacetophenone was not investigated further by us.

The chlorination of 2,5-dihydroxyacetophenone, under similar conditions, resulted in the formation of a trichloro derivative: 2,5-dihydroxy-3,4,6-trichloroacetophenone; m.p. 79–81°C. The IR spectrum in Nujol showed a strong absorption band at 3370 cm^{-1} (OH groups), a sharp band at 1680 cm^{-1} for a —$COCH_3$ group, and benzene ring stretching bands at 1627 and 1598 cm^{-1}. The PMR spectrum showed a sharp singlet at τ 7.72 (3H; $COCH_3$) and two sharp bands at τ 4.4 (H) and τ 1.7 (H) for two phenolic *OH*.

Oxidation of 2,5-diacetoxy-4-chloroacetophenone was carried out by chromium trioxide, yielding 2,5-diacetoxy-4-chlorophenylpyruvic acid (IV), (m.p. 87°C). Its UV spectrum showed λ_{max} at 250 nm (ε 24340). Its IR spectrum showed absorption band at 3205, 2928, 1707 cm^{-1} for carboxyl. Another band appeared at 1754 cm^{-1} for the carbonyls of the acetoxyl groups. The CO of the —COCOOH appeared at 1539 cm^{-1}. The bands at 1608 and 1587 cm^{-1} were due to benzene ring stretching. Pyruvic acid (IV) was oxidized to 2,5-diacetoxy-4-chlorobenzoic acid(V) (m.p. 82°C) with the help of hydrogen peroxide (30%). Its UV absorption bands appeared at λ_{max} 288 and 220 nm. Its IR spectrum showed sharp bands at 1760 and 1700 cm^{-1} for the carbonyls of —$OCOCH_3$ and carboxyl group respectively.

The above diacetoxy acid was esterified with excess of diazomethane which yielded methyl 2, 5-diacetoxy-4-chlorobenzoate (VI) m.p. 62°C. Its UV absorption band showed λ_{max} at 284 nm (ε 2604) and 213 nm (ε 11410). Its IR spectrum showed a sharp band at 1720 and 1770 cm^{-1} for the carbonyls of —$CO.OCH_3$ and $O.CO.CH_3$ groups respectively. The benzenoid ring stretching bands appeared at 1615 and 1590 cm^{-1}.

The PMR spectrum in $CDCl_3$ showed a sharp singlet at τ 6.2 (3H) for —$COOCH_3$ group and the two —$OCOCH_3$ groups appeared as singlets at τ 7.6 (2×3H). The benzenoid protons appeared at τ 3.2 (1H) and τ 2.8 (1H) as sharp singlets, thus confirming structure (VI).

Methyl 2,5-diacetoxy-4-chlorobenzoate (VI) was reduced with lithium aluminium hydride in dry ether which yielded 2,5-diacetoxy-4-chlorobenzyl alcohol (X). Its IR spectrum showed a sharp band at 1750 cm^{-1} for the acetoxyl group.

Compound X was deacetylated with 10% alcoholic KOH yielding 2,5-dihydroxy-4-chlorobenzyl alcohol (XI), m.p. 145°C, which showed identical IR and UV spectra with that of an authentic sample of amudol.

The other route of synthesis involved 2,5-dihydroxybenzoic acid (VII) which showed UV absorption λ_{max} at 318 nm (ε 8260) and a shoulder at 227 nm (ε 13700). It was acetylated in the usual manner with acetic anhydride in pyridine obtaining 2,5-diacetoxybenzoic acid (VIII) (m.p. 62°C) in almost theoretical yield. Its UV absorption spectrum showed λ_{max} at 275 nm (ε 2435) and a shoulder at 308 nm (ε569.4).

The IR spectrum showed the absence of hydroxyl group but showed a strong band at 1770 cm^{-1} for the carbonyl vibration of acetoxyl groups. It showed bands at 3022, 2820, 2750 and 1709 cm^{-1} for the carboxyl group. The benzenoid ring stretching appeared at 1627 and 1587 cm^{-1}.

The PMR spectrum in $CDCl_3$ showed two sharp singlets at τ 7.8 (3H) and τ 7.52 (3H) for the six protons of the two acetoxyl groups. It showed a fine splitting in the benzenoid region. A doublet centred at τ 2.8 appeared for the Hc proton with a coupling constant (J 8 c/s). HB proton showed a double doublet centred at τ 2.7 with *ortho* and *meta* coupling (J 8 and 2 c/s). The proton HA showed a doublet centred at τ 2.1 with *meta* coupling (J 2 c/s). The proton of the carboxyl group appeared as a singlet at τ 0.42. Esterification of 2,5-diacetoxy benzoic acid (VIII) with diazomethane gave methyl 2,5-diacetoxy-benzoate (IX) (m.p. 40°C) as a low melting solid.

The UV absorption bands appeared at λ_{max} 306 nm (ε 1649) and 278 nm (ε 4368). Its IR spectrum showed sharp absorption bands at 1770 and 1748 cm^{-1} for the carbonyl vibrations of acetoxyl group and ester group. The benzene ring stretching appeared at 1614 and 1597 cm^{-1}.

The PMR spectrum in $CDCl_3$ showed two sharp singlets at τ 7.5 (3H) and τ 7.42 (3H) for the protons of the acetoxyl groups. The protons of the methyl ester appeared at τ 5.89 (3H) as a singlet. The benzenoid protons showed very fine splitting with each other as was observed in the previous cases. The proton HB showed a doublet at τ 2.58 (J 9 c/s) indicating an *ortho* orientation; proton Hc showed a double doublet at τ 2.4 with *ortho* and *meta* coupling (J 9 and 2 c/s). Proton HA showed a doublet at τ 1.95 (J 2 c/s), thus confirming the above structure.

Ester (IX) was chlorinated in the same manner as in the case of 2,5-diacetoxyacetophenone. The major product obtained in the reaction was the desired methyl 2,5-diacetoxy-4-chlorobenzoate (VI) (m.p. 62–63°C). This route was found to be more advantageous as the desired intermediate methyl 2,5-diacetoxy-4-chlorobenzoate (VI) was obtained not only in higher yield but also in two steps less than the previous route starting from 2,5-dihydroxyacetophenone. This ester was again reduced with LAH and finally hydrolysed to give good yield of pure amudol.

The work on the synthesis of amudol *in vivo* in the cell-free system is underway and will be reported later.

Experimental

M. ps were taken on Kofler block. IR spectra were recorded on Perkin-Elmer 137 Spectrophotometer and UV spectra were taken in methanol on Beckman DB automatic recording spectrophotometer. PMR spectra were recorded on DSP-double focussing high-resolution varian 60. NMR spectrophotometer, using TMS as an internal stan dard. Petroleum ether had boiling point 60–80°C. Silica-gel used was Marck's PF 254

2,5-*Diacetoxyacetophenone* (*II*)

2,5-Dihydroxyacetophenone (I) (4 g; 0.026 mole) was dissolved in a mixture of anhydrous pyridine (10 ml) and acetic anhydride (20 ml) and refluxed (1 hr). The reaction product was then poured onto crushed ice (50 g) containing HCl (15 ml) when crystals of the acetylated product separated out. The crude product was filtered (suction) and dried over porous plate. Crystallisation from ethyl acetate—petroleum ether gave 2,5-diacetoxyacetophenone (II), m.p. 68°C, 5g (80.5)% (Found: C, 61.09; H, 5.23, O, 33.68. Calc. for $C_{12}H_{12}O_5$: C, 61.02; H, 5.12; O, 33.86%).

2,5-*Diacetoxy*-4-*chloroacetophenone* (*III*)

2,5-Diacetoxyacetophenone (4.3 g, 0.02 moles) was dissolved in 25 ml dry chloroform containing 3 drops of triethylamine and saturated with chlorine gas under ice cooling. The mixture was then irradiated with light from a G.E.C. 1000 W lamp for 4 hr. Glistening yellow crystals separated out. These were filtered (suction) and crystallised from ethanol. 2,5-Diacetoxy-4-chloroacetophenone (III) was obtained as slightly yellow needles, m.p. 54°C, 3.7 g (75.5%). (Found: C, 53.82; H, 4.19; O, 29.23; Cl, 12.78. Calc. for $C_{12}H_{11}O_5Cl$: C, 53.33; H, 4.07; O, 29.65; Cl, 12.95%).

2,5-*Diacetoxy*-4-*chlorophenylpyruvic Acid* (*IV*)

Oxidation of 2,5-*diacetoxy*-4-*chloroacetophenone* (*III*). —2,5-Diacetoxy-4-chloroacetophenone (3 g, 0.011 mole) was dissolved in a mixture of acetic acid (2 ml) and H_2SO_4 (d 1.84; 5 ml) and chromium trioxide (1.3 g) was added in portions under shaking. As soon as the reaction was over (4 hr) a little methyl alcohol was added to completely remove any unreacted chromium trioxide, the mixture was shaken for additional ½ hr and extracted with ethyl acetate several times. The combined extract was repeatedly washed with small portions of water, dried (Na_2SO_4) and filtered. Removal of solvent gave 2,5-diacetoxy-4-chlorophenylpyruvic acid (IV) which was recrystallised from ethyl acetate and petroleum ether; light yellow microscopic plates, m.p. 87°C, 2.3 g (69%). (Found: C, 48.25; H, 3.17; O, 38.02; Cl, 10.56%. Calc. for ($C_{12}H_9ClO_7$): C, 48.00; H, 3.00; O, 37.25; Cl, 11.75%.)

2,5-*Diacetoxy*-4-*chlorobenzoic Acid* (*V*)

Oxidation of 2,5-*diacetoxy*-4-*chlorophenylpyruvic acid* (*IV*).—2,5-Diacetoxy-4-chlorophenylpyruvic acid (IV) (2 g, 0.006 mole) was dissolved in acetic acid (10 ml) and H_2O_2 (30%, 5 ml) was added to it. The reaction mixture was stirred for 4 hr. The excess of the oxidant was removed by adding 10% Pd-C (20 mg). It was filtered, the solvent was removed, water was added and the mixture extracted with ethyl acetate. The extract was dried

(Na_2SO_4), filtered and on removal of the solvent yielded pure 2,5-diacetoxy-4-chlorobenzoic acid (V), m.p. 82°C, 1.2 g (66.66%). (Found: C, 48.72; H, 3.14; O, 35.1; Cl, 13.04%. Calc. for $C_{11}H_9ClO_6$: C, 48.8; H, 3.33; O, 35.5; Cl, 12.90%).

Methyl 2,5-diacetoxy-4-chlorobenzoate (VI)

2,5-Diacetoxy-4-chlorobenzoic acid (V) (1.44 g, 0.005 mole) was esterified with excess of diazomethane. Removal of ether gave methyl 2,5-diacetoxy-4-chlorobenzoate (VI) as an oil which turned to solid on standing. Colourless prismatic needles from chloroform, m.p. 62°C, (1.5 g (92.3%). (Found: C, 50.03; H, 3.44; O, 33.82; Cl, 12.71%). Calc. for $C_{12}H_{11}O_6Cl$: C, 50.24; H, 3.84; O, 33.08, Cl, 12.84%.)

2,5-Diacetoxy-4-chlorobenzyl alcohol

(a) *LAH Reduction of Methyl 2,5-Diacetoxy-4-chlorobenzoate.*—Methyl 2,5-diacetoxy-4-chlorobenzoate, (300 mg, 0.011 mole) was dissolved in anhydrous ether and LAH (300 mg) was added in small portions at intervals of 15 min over a period of 2 hr during reflux. The excess of LAH was decomposed by the careful addition of ethyl acetate, and the complex was decomposed with 2N HCl and extracted with ethyl acetate. The extract was dried (Na_2SO_4) and the solvent was removed. A semisolid residue (198 mg) of 2,5-diacetoxy-4-chlorobenzyl alcohol (X) contaminated with three other products was obtained, as shown by TLC (solvent system: ethyl acetate–petroleum ether 1:1).

(b) *Decetylation of the Above Reduced Product.*—The product (198 mg) was taken up in alcoholic KOH (5%, 10 ml) and allowed to stand (6 hr) at room temperature. The solvent was removed, water was added, the residue acidified with 2N HCl, extracted with ethyl acetate, and dried (Na_2SO_4). Removal of solvent gave a semisolid residue from which amudol was separated by preparative thin layer chromatography using kieselgel as absorbent and ethyl acetate and petroleum ether (1:1) as solvent system. The compound was eluted with methanol and then purified by dissolving in chloroform. On removal of the solvent, pure amudol, m.p. 146–147°C was obtained which was recrystallised from ether–petroleum ether, 25 mg, m.p. 147°C.

2,5-Dihydroxy-3,4,6-trichloroacetophenone

2,5-Dihydroxyacetophenone (300 mg, 0.002 mole) was dissolved in chloroform (50 ml) containing a drop of triethylamine. Chlorine was passed in the ice-cold solution till it was saturated and the mixture was exposed to the light of a 1000 W G.E.C. electric bulb (4 hr). Removal of the solvent gave 2,5-dihydroxy-3,4,6-trichloroacetophene light yellow prismatic needles from ethyl acetate, m.p. 79–81°C (250 mg, 50%). (Found: C, 37.42; H, 3.07; O, 18.19; Cl, 41.32%. Calc. for $C_8H_5Cl_3O_3$: C, 37.79; H, 2.99; O, 18.89; Cl, 41.33%.)

2,5-Diacetoxybenzoic Acid (VIII)

Acetylation of 2,5-Dihydroxybenzoic Acid (VII).—2,5-Dihydroxybenzoic acid (4 g) was dissolved in pyridine (5 ml) and acetic anhydride was added to it (15 ml). The reaction mixture was heated on a water bath for ½ hr and left overnight in the dark. The reaction mixture was poured on to crushed ice and extracted with ethyl acetate. The ethyl acetate extract was washed with HCl (2N) and then with water. The solvent was removed, yielding 2,5-diacetoxybenzoic acid (VIII) m.p. 62°C, 5 g (89.0%).

Methyl 2,5-Diacetoxybenzoate (IX)

Esterification of 2,5-Diacetoxy Benzoic Acid.—2,5-Diacetoxybenzoic acid (5 g) was reacted with excess of diazomethane in ether which gave methyl 2,5-diacetoxybenzoate, m.p. 40°C, in theoretical yield.

Methyl 2,5-diacetoxy-4-chlorobenzoate (VI)

Chlorination of Methyl 2,5-Diacetoxybenzoate.—Methyl 2,5-diacetoxy benzoate (5 g, 0.019 mole) was dissolved in chloroform (25 ml) containing 0.2 ml of triethylamine and then chlorine was passed for 5 min. The reaction mixture was irradiated by a G.E.C. 1000 W electric bulb, for 4 hr. The solvent was removed, yielding a semisolid residue which contained the required methyl 2,5-diacetoxy-4-chlorobenzoate as a major component. It was purified through preparative thin layer chromatography using ether–petroleum ether (1:2) as the solvent system. The band with R_f value 0.76 was eluted with chloroform giving pure methyl 2,5-diacetoxy-4-chlorobenzoate, m.p. 62–63°C, 2.3 g (45.6%).

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