Pakistan J. Sci. Ind. Res., Vol. 13, No. 3, October 1970

STUDIES IN THE BIOCHEMISTRY OF MICROORGANISMS

Part XI.—Isolation of Tajixanthone, Shamixanthone, Ajamxanthone, Shahenxanthone, Najamxanthone, Radixanthone and Mannitol from Mycelium of Aspergillus stellatus, Curzi

Ahmad Kamal, Shaheen A. Husain, Radia Noorani, Najma Murtaza,
I.H. Qureshi and Asaf A. Qureshi

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

(Recevied February 13, 1970)

Isolation of six pigments are described: *tajixanthone*, m.p. 157–158°C, $C_{25}H_{26}O_6$; *shamixanthone*, m.p. 153–154°C, $C_{25}H_{25}O_5$; *ajamxanthone*, m.p. 132–134°C, $C_{25}H_{24}O_4$; *shahenxanthone*, m.p. 163–164°C, $C_{25}H_{28}O_7$; *najamxanthone*, m.p. 180–182°C, $C_{26}H_{28}O_8$; *radixanthone*, m.p. 202–203°C, $C_{26}H_{30}O_9$ and mannitol from *Aspergillus stellatus*, Curzi.

In a previous communication[1] the isolation of terrein and kojic acid from metabolic solution of *Aspergillus stellatus*, Curzi was described. This paper deals with the isolation and characterization of six golden yellow pigments and mannitol from the mold mycelium.

The mold was grown on Czapek–Dox medium enriched with carrot extract and diammonium tartrate as nitrogen source.[1] The separated mycelium, dried and finely powdered, was extracted with petroleum ether and finally with ethanol. Removal of the solvent from the petroleum ether fraction gave a purple coloured viscous oil which on extraction with methanol gave crystalline golden yellow residue (m.p. 140–46°C). The methanol-insoluble, purple oily residue is being investigated and our findings will be reported later.

The ethanol extract gave a sugar, $C_6H_{14}O_6$ (m.p. 164–65°C) identified as mannitol (m.p. 166°C) and further confirmed through its hexa-acetate (m.p. 119–120°C).

The golden yellow, crystalline petroleum ether-soluble fraction, m.p. 140–46°C, when subjected to preparative thin layer chromatography over Keiselgel G, with benzene–ether (95:5) as solvent system, gave six golden yellow pigments. These pigments have not been described before and have been given the following names for ease of characterization and discussion: *Tajixanthone*, *Shamixanthone*, *Ajamxanthone*, *Shahenxanthone*, *Najamxanthone* and *Radixanthone*.

All these pigments have UV absorption bands in the range of 240–390 mμ and showed λ_{max} at 386, 294, 273 and 240 mμ in each case. These UV absorption bands were strongly suggestive of the presence of a xanthone nucleus in the molecules of these pigments. For instance, 1,2-dihydroxyxanthone[2] shows λ_{max} at 385 mμ, 290 mμ, 261 mμ, and 244 mμ. Similarly 1,4-dihydroxyxanthone shows λ_{max} at 390 mμ, 299 mμ, 263 mμ and 229 mμ, and mangostin[3] and its derivatives, which have the xanthone structure, showed the same four UV absorption bands in the same range of 240–400 mμ.

Tajixanthone.—Tajixanthone, the major fraction, was obtained as very light fluffy golden yellow needles and analysed for $C_{25}H_{26}O_6$. The molecular formula was further confirmed from its high resolution mass spectrum (found: 422.1776; calc. 422.1784). The UV absorption spectrum showed λ_{max} at 383 mμ (ε 4094), 292 mμ (ε 15870), 270 mμ (ε 37450) and 262 mμ (ε 34930), 248 mμ (ε 27640) and 235 mμ (ε 33670). The IR spectrum showed the presence of —OH (3500 cm^{-1}), $CH_2{=}CH$—(2925 cm^{-1}), >C=O (1635 cm^{-1}), benzene ring stretching (1600 cm^{-1}, 1568 cm^{-1}), C—methyl (1470 cm^{-1}). The PMR spectrum in deuterochloroform showed four sharp singlets at τ 8.70 (3H); τ 8.55 (3H); τ 8.20 (3H) and τ 7.78 (3H) each indicating three C—methyl groups, one of them (at τ 8.20) located on the benzene ring. Another sharp singlet at τ 6.98 (3H) was indicative of either a C—CH_3 or an O—CH_3. A multiplet centred at τ 7.28 (1H) indicated a methine proton. A doublet appearing at τ 5.57 (2H) indicated a methylene group due to AB_2 system (J_{AB_2} 3 c/s). There were two broad singlets at τ 5.18 (1H) and τ 5.17 (1H) each for a methine proton adjacent to a double bond. An ill-defined quartet was centred at τ 4.58 (1H) due to AB and AX system (J_{AB} 3 c/s; J_{AX} 4 c/s). In the benzenoid region there were two doublets (2H), appearing at τ 3.24, τ 2.48 (J 10 c/s), indicating the presence of two protons *ortho* to each other. Another sharp singlet appearing at τ 2.69 (1H) showed the presence of an isolated proton on a second benzene ring of the xanthone nucleus of the molecule. A sharp singlet and a doublet centred at τ −0.76 (1H) and 5.02 (1H) due to AX system (J_{AX} 4 c/s), which disappeared on deuteration, indicated the presence of one phenolic and one hydroxyl group, respectively.

The above PMR data accounts for all the twenty-six protons present in the molecule of tajixanthone, $C_{25}H_{26}O_6$.

Tajixanthone gave positive iodoform test (—CHOH or —$COCH_3$) and brown colour with alcoholic ferric chloride (phenolic —OH). It was optically active, $[\alpha]_D^{27°}$ −80 (0.2% in chloroform). It gave a positive Perkin-γ-pyrone test.

Shamixanthone.—Shamixanthone, obtained in golden yellow, silky fluffy needles, analysed for $C_{25}H_{26}O_5$. The molecular formula was further confirmed from its high resolution mass spectrum (found: 406.1736, calc. 406.1780). The UV absorption spectrum showed λ_{max} at 394 mμ (ε 3179), 297 mμ (ε 2716), 272 mμ (ε 11610), 262 mμ (ε 10390), 250 mμ (ε 84650) and 238 mμ (ε10160). The IR absorption spectrum showed the presence of phenolic —OH (3500 cm^{-1}); CH_2 =CH—(2945 cm^{-1}), >C=O (1637 cm–1); benzene stretching (1600 cm^{-1} and 1575 cm^{-1}), C—methyl (1470 cm^{-1}).

The PMR spectrum in deuterochloroform gave a sharp singlet at τ 8.18 (6H) representing *gem*-dimethyl groups. Another singlet at τ 8.20 (3H) indicated a methyl group on a benzene ring and a sharp singlet appeared at τ 7.64 (3H), for methyl group on a benzene ring with a hetero atom next to it. Then there was a multiplet centred at τ 7.26 (1H) for a methine proton. There was a doublet and triplets centred at τ 6.43 (1H) and τ 6.55 (1H) due to AX_2 system, indicating the presence of CH_2OH group in the molecule. A doublet centred at τ 5.57 (2H), due to AB_2 system (J_{AB_2} 3 c/s). Two broad singlets appeared at τ5.33 (1H) and τ 5.14 (1H) due to two methine protons in different environments. A broad multiplet centred at τ4.60 (2H), could be due to two methine protons in similar environment or one methylene group. Two doublets centred at τ 3.2 (1H) and τ 2.5 (1H) due to two benzenoid protons in *ortho* orientation; (J 10 c/s). A singlet appeared at τ 2.6 (1H) indicative of a benzenoid proton in the second benzene ring of the xanthone moiety. Two sharp singlets appearing at τ 9.42; 0.75 (2H; alcoholic acid and phenolic —OH; disappearing on deuteration), finally accounted for all the twenty-six protons of shamixanthone $C_{25}H_{26}O_5$.

Shamixanthone gave positive Perkin-γ-pyrone test. It gave a brown colour with ferric chloride.

Ajamxanthone.—Ajamxanthone, obtained in golden yellow, silky fluffy needles, analysed for $C_{25}H_{24}O_4$. The molecular formula was further confirmed from its high resolution mass spectrum (found: 388.1681; calc. 388.1675).

UV absorption spectrum showed bands at λ_{max} 389 mμ (ε 1007), 292 mμ (ε 11740), 269 mμ (ε 44180), 261 mμ (ε 40410), 247 mμ (ε 30110) and 235 mμ (ε 33480). IR spectrum showed absorption bands for three phenolic —OH (3475 cm^{-1}), CH_2=CH—(2910 cm^{-1}), >CO (1645 cm^{-1}), benzene ring stretching (1600 cm^{-1} and 1575 cm^{-1}), C—methyl (1475 cm^{-1}).

Ajamxanthone gave a positive Perkin-γ-pyrone test. It also gave a positive ferric chloride test.

Shahenxanthone.—Shahenxanthone analysed for $C_{25}H_{28}O_7$. Its high resolution mass spectrum further confirmed its molecular formula (found: 440.2060; calc. 440.2072).

UV absorption spectrum showed λ_{max} at 389 mμ (ε 6926), 292 mμ (ε 99490); 271 mμ (ε 36440) and 263 mμ (ε 33700); 249 mμ (ε 25880) and 238 mμ (ε 28410). IR absorption spectrum showed the presence of phenolic OH (3470 cm^{-1}) and alcoholic—OH (3420 cm^{-1}), CH_2=CH—(2925 cm^{-1}), >CO (1650 cm^{-1}), benzene stretching (1605 cm^{-1} and 1595 cm^{-1}), C—CH_3 (1475 cm^{-1}).

The PMR spectrum in deuterochloroform showed an ill-defined doublet at τ8.88 (3H) due to AB_3 system (J_{AB_3} 7 c/s). There were two superimposed signals at τ 8.73 (6*H*; *gem*-dimethyl). Two more singlets appeared at τ 8.1 (3H) and τ 7.67 (3H) for two more C—methyl groups. An ill-defined multiplet appeared at τ 8.28 (1H) due to one methine proton. Two multiplets appeared at τ 7.20 (1H) and τ 6.52 (1H) for two separate methine protons.

A fine doublet appeared at τ 5.64 (2H) indicating a methylene next to a =CH—(J_{AB_2} 2 c/s). Two broad singlets appeared at τ 5.42 (1H) and τ 5.22 (1H) showing the presence of two methine protons more or less in the same environment. Two multiplets appeared at τ 4.58 (1H) and τ 5.08 (1H) due to two hydroxyl groups, which disappeared on deuteration. The spectrum showed the same pattern of three benzenoid protons as observed in the case of tajixanthone and shamixanthone, viz. two separate doublets at τ 3.28 (1H; J 8.5 c/s) and τ 2.45 (1H; J 8.5 c/s) indicating that these two protons were in *ortho* position to each other. The third proton showed a singlet at τ 2.78 (1H; isolated benzenoid proton). The twenty-eighth proton appeared at τ−1.5 (O*H*) which disappeared on deuteration.

It gave a positive Perkin-γ-pyrone test and with ferric chloride solution it gave a brown colour.

Najamxanthone.—Najamxanthone analysed for $C_{26}H_{28}O_8$. Its high resolution mass spectrum further confirmed its molecular formula (found: 468.2478; calc: 468.2417). Its UV absorption spectrum showed λ_{max} at 388 mμ (ε 3979), 294 mμ (ε 14460), 272 mμ (ε 44690) and 264 mμ (ε 40290), 249 mμ (ε 34290) and 238 mμ (ε 39290). IR absorption showed the presence of a phenolic hydroxyl (3450 cm^{-1}) and an alcoholic hydroxyl (3390 cm^{-1}), $CH_2{=}CH$—(2990 cm^{-1}, 2930 cm^{-1}), >CO (1648 cm^{-1}), benzene ring stretching (1600 cm^{-1} and 1590 cm^{-1}) and C—CH_3 (1480 cm^{-1}).

PMR spectrum in deuterochloroform showed a doublet centred at τ 8.87 (3H; due to AB_3 system; J_{AB_3} 7 c/s) indicating the presence of a —C*H*$_3$ next to a >CH—. There was a sharp singlet at τ 8.78 (6H) due to *gem*-dimethyl. Two more sharp singlets appeared at τ 8.20 and τ 7.70 for C—C*H*$_3$ groups, the former located on a benzene ring. There was a multiplet at τ 8.30 (1H) due to a methine proton. A series of multiplets appeared between the range of τ 7.57 and τ 6.23 accounting for three protons. A sharp doublet appeared at τ 5.25 (2H; J_{AB_2} 3 c/s) due to a methylene group. Two broad singlets at τ 5.65 (1H) and τ 5.25 (1H), indicated the presence of methine protons. An ill-defined doublet which appeared at τ 5.10 (1H) and a similarly ill-defined quartet centred at τ 4.6 (1H) accounted for one hydroxyl group (which disappeared on shaking with deuterium oxide) and one methine proton respectively. The signals for two benzenoid protons appeared in the same pattern as observed in the previous cases. They were centred as doublets at τ 3.28 and τ 2.42, showing *ortho* coupling (*J* 8 c/s) and as a sharp singlet at τ 2.75 for one isolated benzenoid proton. As in all previous cases, it gave a positive Perkin-γ-pyrone test and with ferric chloride it gave a brown colour.

Radixanthone.—Radixanthone was isolated in a very minute quantity. It melted at 202–203°C and its mass spectral analysis showed its molecular ion to be 486^+ and its molecular formula $C_{26}H_{30}O_9$. It has the same UV and IR spectra as those of the previously described pigments.

Work on the consttiution and the biogenesis of these six pigments is in progress and will be reported later.

Experimental

Melting points were taken on Kofler block and are uncorrected. UV spectra were measured in ethanol (95%) on Beckman D.B. spectrophotometer. IR spectra were determined with a Perkin-Elmer 137 instrument in KBr unless otherwise stated. PMR spectra were recorded on 60 Mc/s on Perkin-Elmer R-10 Varian A-60 and Varian HA 100 containing TMS as internal reference standard. Mass spectra were measured on AEI MS9 at 70e V. Petroleum ether used had b.p. 65–86°C. Microanalyses were by Dr. A. Bernhardt, Muelheim, West Germany.

Culture Conditions

Aspergillus stellatus Curzi (C.M.I. No: IMI, 112543) was first grown on ordinary Czapek–Dox medium in test tubes and then inoculated at 24°C for 9 days. This 9-day old culture was used for inoculating flasks containing culture medium. The medium used was composed of glucose 50 g, K_2HPO_4 1.0 g, KCl 0.5 g, $MgSO_4.7H_2O$ 0.5 g, $FeSO_4.7H_2O$ 0.01 g, and aqueous carrot extract 1 litre. In a typical batch five (1-l.) conical flasks were taken, each containing 340 ml of the above medium and autoclaved at 10 lb pressure for 20 min. Diammonium tartrate solution (7.56 g/l) was sterilized and 10 ml of it was added asceptically to each of the flasks. This precaution was taken to avoid the discoloration of the medium during sterilization. The flasks having culture medium (pH 4.5) were inoculated with 9-day old tube culture of *Aspergillus stellatus* and incubated at 24°C for 17 days. A thin mycelial felt developed which was green in colour on the top and purplish brown on the reverse side. After 17 days the mycelium was removed by filtration and the broth (1475 ml) from 42 flasks was extracted with ethyl acetate which on concentration afforded two crystalline products, terrein and kojic acid as reported in the previous communication.[1] The mycelium was dried in the oven at 55–60°C for 4 days. The dried mycelium (50 g from 5 flasks) was powdered and extracted in a Soxhlet extractor with petroleum ether (30 hr). The solvent was removed and the semisolid residue was extracted with methanol. The methanol-insoluble fraction yielded a purple oil (5.1 g) which is being investigated. The methanol-soluble material yielded a mixture of golden yellow pigments which were separated into the following six compounds by preparative thin layer chromatography, using benzene–ether (95:5) as solvent system:

Compound	R_f	M.p. °C	Mol. formula
Tajixanthone	0.27	157–58	$C_{25}H_{26}O_6$
Shamixanthone	0.49	153–54	$C_{25}H_{26}O_5$
Ajamxanthone	0.42	132–34	$C_{25}H_{24}O_4$
Shahenxanthone	0.16	163–64	$C_{25}H_{28}O_7$
Najamxanthone	0.12	180–82	$C_{26}H_{28}O_8$
Radixanthone	0.00	202–203	$C_{26}H_{30}O_9$

The mycelium was further extracted with ethyl acetate, ether and chloroform respectively and yielded the same pigments as were isolated from petroleum ether. Finally, the mycelium was extracted with ethanol for 30 hr. On removal of the solvent, the extract yielded a crystalline compound, m.p. 165°C, which analysed for $C_6H_{14}O_6$. Its IR spectrum showed absorption bands at 3356 cm^{-1} (OH free), 2985 cm^{-1} ($CH_2{=}CH$), 1460, 1433, 1379, 1361, 1325, 1266, 1198, 1086, 1020, 955, 925 and 885 cm^{-1}. PMR spectrum of this compound showed a multiplet at τ 7.5 (6H, 6 × OH), a doublet at τ 5.88 (4H, $2 \times CH_2$; due to AB_2 system: J_{AB_2} 3 c/s) and one more multiplet centred at τ 5.65 (4*H*, methine protons). It was found to be mannitol (m.p. 164–65°C, R_f 0.89, and yield 0.3 g) by comparison with paper and thin layer chromatography (solvent system, ethanol–acetic acid–water 4:1:1), against an authentic sample which showed identical R_f value. The mixed m.p. was also undepressed.

Hexa-acetate of Mannitol

Mannitol (100 mg) was dissolved in acetic anhydride (1.5 ml) and anhydrous sodium acetate (100 mg) was added into it and heated for 15 min. The reaction mixture was poured over ice. The precipitate was filtered and washed with ice-cold water. The precipitate on crystallization from ethanol yielded colourless fine needles of hexa-acetate of mannitol, m.p. 119–120°C, undepressed by mixing with an authentic sample of hexa-acetate of mannitol, m.p. 120°C.

Tajixanthone

Tajixanthone obtained by TLC was crystallized several times from ethanol and gave golden yellow needles (260 mg, m.p. 157–58°C, $[\alpha]_D^{27°C}$ −80 (0.2% solution in chloroform), which analysed for $C_{25}H_{26}O_6$. (Found: C, 71.34; H, 6.55; O, 22.26%. $C_{25}H_{26}O_6$: requires C, 71.07; H, 6.20; O, 22.72%.) Its IR spectrum (in KCl) showed sharp bands at 3425 (OH), 2925, 2850 ($CH_2{=}CH{-}$), 1635 (C=O), 1600, 1570 (benzene ring stretching), 1470, 1430, ($-CH_3$) 1359, 1250, 1200, 1001, 900 and 855 cm^{-1}.

It gave brown colour with ferric chloride, and positive Perkin test for γ-pyrone.

Perkin-γ-Pyrone Test.—Tajixanthone (1 mg) when dissolved in acetic acid (5 ml) yielded an orange colour on the addition of one drop of concentrated sulphuric acid.

Shamixanthone

Shamixanthone obtained by TLC was crystallized from methanol or ethanol, gave fluffy needles (75 mg), m.p. 152–53°C). It analysed for $C_{25}H_{26}O_5$. (Found: C, 73.86; H, 6.57; O, 19.4%. $C_{25}H_{26}O_5$ requires C, 73.86; H, 6.45; O, 19.68%.)

It was soluble in most of the organic solvents. Its IR spectrum showed strong bands at 3500 cm^{-1} (OH), 1640 cm^{-1} (C=O), 1600 and 1577 cm^{-1} (benzene ring stretching) and other bands were at 1475, 1425, 1352, 1295, 1265, 1245, 1198, 1120, 1080, 1050, 900, 860, 825, 810, 755 and 730 cm^{-1}. It showed positive test for γ-pyrone It gave brown colour with alcoholic ferric chloride solution showing the presence of phenolic hydroxyl group.

Ajamxanthone

Ajamxanthone obtained by preparative TLC was crystallized from chloroform–ether and yielded golden yellow needles (25 mg, m.p. 132–133°C). It analysed for $C_{25}H_{24}O_4$. (Found: C, 77.50; H, 6.43; O, 16.67%. $C_{25}H_{24}O_4$ requires: C, 77.30; H, 6.23; O, 16.47%.)

It gave brownish colour with ferric chloride solution and was soluble in most of the organic solvents. It gave an orange ring with acetic and sulphuric acids indicating the presence of γ-pyrone. Its IR spectrum (in KBr) showed bands at 3500 cm^{-1} (OH), 1640 cm^{-1} (C=O), 1600 and 1575 cm^{-1} (benzene ring stretching) and other bands were at 1475, 1460, 1430, 1360, 1295, 1265, 1245, 1198, 1120, 1080, 1050, 900, 860, 826, 810, 755 and 730 cm^{-1}.

Shahenxathone

When crystallized from ethanol, shahenxanthone obtained by preparative TLC yielded yellow fluffy plates (40 mg, m.p. 163–64°C) and analysed for $C_{25}H_{28}O_7$. (Found: C, 68.4; H, 6.22; O, 25.5%. $C_{25}H_{28}O_7$ requires: C, 68.17; H, 6.41; O, 25.42%.)

It was soluble in ether, ethyl acetate, chloroform, acetone, sparingly soluble in ethanol and methanol and insoluble in carbon tetrachloride and water. It gave a positive test for γ-pyrone with the formation of an orange ring with acetic acid and sulphuric acid. Its IR spectrum (in KBr) showed sharp bands at 3495, 3425 cm^{-1} (OH), 2945 cm^{-1} (—CH=CH$_2$), 1650 cm^{-1} (C=O), 1602 and 1580 cm^{-1} (benzene ring stretching). Other bands appeared at 1475, 1427, 1380, 1352, 1248, 1198, 1085, 1050, 900, 830 and 950 cm^{-1}.

Najamxanthone

Najamxanthone obtained by TLC was crystallized from methanol yielding golden yellow needles (25 mg, m.p. 180–182°C). It analysed for $C_{26}H_{28}O_8$. (Found: C, 66.57; H, 6.45; O, 27.41%. $C_{26}H_{28}O_8$ requires: C, 66.66; H, 6.02; O, 27.32%.)

It was soluble in most of the organic solvents. It also gave positive test for γ-pyrone and brown colour with ferric chloride solution. Its IR spectrum (in KBr) showed bands at 3450 cm^{-1} (OH), 2980 cm^{-1} (—CH—CH$_2$), 1640 cm^{-1} (>C=O), 1600 and 1585 cm^{-1} (benzene ring stretching). It also showed similar bands as were observed in the previously described compounds.

Acknowledgement.—We are indebted to Professor A.I. Scott, D.Sc., Sterling Chemistry Laboratories, Yale University, Conn., U.S.A., for granting facilities for the use of PMR and mass spectrometers and Dr. W. J. McMurrary for the computerised high resolution mass spectral data.

References

1. I.H. Qureshi, A. Kamal, R. Noorani, S. Aziz and S.A. Husain, Pakistan J. Sci. Ind. Res., **11,** 367 (1968).
2. O.R. Gottlieb, M. Magalhaes, M.O. da Silva Pereira, A.A. Lins Misquita, D. De. Barros Correa and G.G. De Oliveria, Tetrahedron, **24,** 1601 (1968).
3. P. Yates and G.H. Stout, J. Am. Chem. Soc., **80,** 1691 (1958).
4. M.L. Wolfran, E.E. Dickey, P. McWain, A. Thompson, J.H. Looker, O.M. Windrath and F. Komitsky, J. Org. Chem., **29,** 689 (1964).