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SYNTHESIS OF 7-HYDROXY-6, 2′, 4′, 5′-TETRAMETHOXYISOFLAVONE

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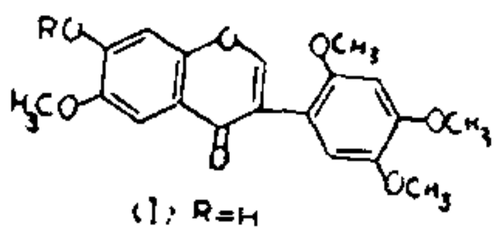
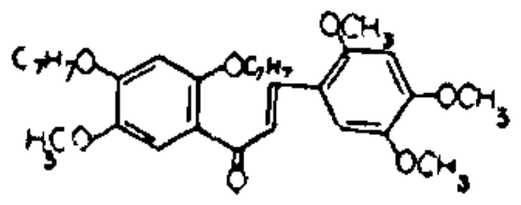
ABSTRACT

Synthesis of 7-hydroxy-6, 2′, 4′, 5′-tetramethoxyisoflavone (I) isolated from *Pterodon apparicoi* has been carried out using the epoxide (IV) of the chalkone (III).

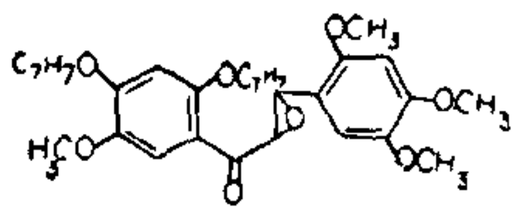
AN isoflavone[1] isolated from *Pterodon apparicoi* was considered to be 7-hydroxy-6, 2′, 4′, 5′-tetramethoxyisoflavone (I) which was later synthesised by Krishnamurti *et al.*[2]. This communication reports the synthesis of the isoflavone (I) and its 7-methyl ether (II) by method that is more convenient than the earlier method[2] and is based on the biogenetic considerations[3–6] which suggest chalkones to be the precursors for such compounds. For this purpose 2′, 4′-dibenzyloxy-2, 4, 5, 5′-tetramethoxychalkone (III) obtained by the condensation of 2, 4, 5-trimethoxybenzaldehyde[7] with 2, 4-dibenzyloxy-5-methoxy-acetophenone,[8] was used as the starting compound. The chalkone (III), when treated with alkaline hydrogen peroxide, gave its epoxide (IV) which on treatment with boron trifluoride-etherate underwent rearrangement as reported[9–13] earlier in the similar cases, to yield the corresponding α-formyldesoxybenzoin which, when directly treated with hydrochloric acid in acetic acid, gave the required isoflavone (I) that was methylated to obtain its 7-methyl ether (II). The synthetic isoflavones (I and II) agreed with the natural sample of the compound and its methyl ether.



(I) R=H
(II) R=CH$_3$



(III)



(IV)

EXPERIMENTAL

2′, 4′-Dibenzyloxy-2, 4, 5, 5′-tetramethoxychalkone (III):

A solution of 2, 4-dibenzyloxy-5-methoxyacetophenone[8] (2·0 g) and 2, 4, 5-trimethoxybenzaldehyde[7] (1·0 g) in alcohol (30 ml) was treated with potassium hydroxide (1·5 g in 1·5 ml of water) and the resulting reaction mixture was left at room temperature for 48 hrs. On acidification with hydrochloric acid it gave a yellow solid that was filtered and washed. It crystallised from ethanol to give the chalkone (III) as yellow needles (1·5 g), m.p. 146–47° (Found: C, 72·9; H, 5·5. $C_{33}H_{32}O_7$ required C, 73·3; H, 5·9%). It did not give any colouration with alcoholic ferric chloride.

2′, 4′-Dibenzyloxy-2, 4, 5, 5′-tetramethoxychalkone epoxide (IV):

A solution of the chalkone (III) (1·0 g) in a mixture of acetone (60 ml) and methanol (10 ml) was treated with aqueous sodium hydroxide (2 ml, 8%) and then hydrogen peroxide (2 ml, 30%) was added. The resulting reaction mixture was left overnight at room temperature. On dilution with water (100 ml), the epoxide (IV) separated out as light yellow coloured solid that was filtered, washed with water and then dried. It crystallised from chloroform-petroleum ether as light yellow needles (0·8 g), m.p. 120–21° (Found: C, 71·1; H, 5·6. $C_{33}H_{32}O_8$ required C, 71·2; H, 5·8%).

7-Hydroxy-6, 2′, 4′, 5′-tetramethoxyisoflavone (I):

A solution of the epoxide (IV) (0·5 g) in dry benzene (30 ml) was treated with boron trifluoride-etherate (1·0 ml) and then stirred for 1 hr. The reaction mixture was extracted with ether and ethereal layer was washed with water. Removal of the solvent from the extract yielded a residue that was dissolved in glacial acetic acid (100 ml) and then treated with concentrated hydrochloric acid (3 ml). The resulting solution was heated on a boiling water-bath for 2 hrs., cooled and then diluted with water (180 ml). This was extracted with ether and then ethyl acetate. Removal of the solvents from the combined extracts gave a residue which was treated with aqueous sodium carbonate (10%). The sodium carbonate soluble fraction on acidification with hydrochloric acid yielded the required isoflavone (I) that was extracted with ethyl acetate. The isoflavone (I) thus obtained was purified by preparative TLC and then crystallised from ethyl acetate as colourless needles (50 mg), m.p. 202–4° (Found: C, 63·22; H, 5·00. $C_{19}H_{18}O_7$ required C, 63·68; H, 5·06%). U.V. (CH_3OH) 255, 305, 325 nm. No characteristic shift with NaOAc.

6, 7, 2′, 4′, 5′-Pentamethoxyisoflavone (II):

A solution of the isoflavone (I) (30 mg) in a mixture of dry ether (15 ml) and dry chloroform (5 ml) was treated with an ethereal solution of diazomethane and then left in the ice-chest for 24 hrs. Removal of the solvent gave a residue that crystallised from ethyl

acetate-petroleum ether as colourless crystalline solid, m.p. 172–73° (Found: C, 64·32; H, 5·12. $C_{20}H_{20}O_7$ required C, 64·5; H, 5·4%). U.V. (CH_3OH) 255, 305 nm. N.M.R. (δ, $CDCl_3$, TMS as internal standard): 3·76–3·90 (15 H, 5 × $-OCH_3$), 6·58 (1H, s, C_3–H), 6·81 (1H, s, C_6–H), 6·87 (1H, s, C_8–H), 7·59 (1H, s, C_5–H), 7·81 (1H, s, C_2–H).

ACKNOWLEDGEMENT

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1. Galina, E. and Gottlieb, O. R., *Phytochem.*, 1974, **13**, 2593.
2. Krishnamurty, M. and Seshagiri, S. N., *Indian J. Chem.*, 1976, **14B**, 951.
3. Grisebach, H. and Patschke, L., *Ber. dt. Chem. Ges.*, 1960, **93**, 2326.
4. —, *Chemistry of Natural Phenolic Compounds*, Pergamon Press, London (Ed. Ollis, W. D.), 1961, p. 59.
5. Pelter, A., Bradschaw, J. and Warren, R. F., *Phytochem.*, 1971, **10**, 835.
6. Hahlbrock, K. and Grisebach, H., *The Flavonoids*, Chapman and Hall, London (Eds. Harborne, J. B., Mabry, T. J. and Mabry, H.), 1975, p. 866.
7. Govindachari, T. R., Nagarajan, K. and Parthasarthy, P. C., *J. Chem. Soc.*, 1957, 548.
8. Jain, A. C., Rohtagi, V. K. and Seshadri, T. R., *Indian J. Chem.*, 1973, **11**, 98.
9. Grover, S. K., Jain, A. C. and Seshadri, T. R., *Ibid.*, 1963, **1**, 517.
10. Bhrara, S. C., Jain, A. C. and Seshadri, T. R., *Tetrahedron*, 1964, **20**, 1141.
11. —, — and —, *Ibid.*, 1965, **21**, 963.
12. Jain, A. C., Sarpal, P. D. and Seshadri, T. R., *Indian J. Chem.*, 1965, **3**, 369.
13. House, H. O., *J. Am. Chem. Soc.*, 1956, **78**, 2298.

IMMUNOCYTOCHEMICAL TECHNIQUES FOR LOCALIZATION OF C_3 COMPONENT OF THE COMPLEMENT

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C_3 COMPONENT of the complement is synthesized and secreted in the blood by visceral organs such as liver, spleen, etc.[1,2]. C_3 levels are measured in the blood serum mainly by immunoprecipitation methods[3]. However, no method is available for localization of this component at the site of synthesis in the cells. Nevertheless, C_3 component has been shown by ferritin labelling on the erythrocyte membrane involving the formation of immune complexes[4]. In this communication we describe methods for the localization of C_3 component in the tissues using monospecific anti-C_3 sera raised in rabbits.

Cross reactivity of sera from different animals to anti-human C_3 was checked by Ouchterlony technique. The central well was loaded with anti-human C_3 serum (anti β_1 C/β_1 A globulin; Behring Werke, West Germany). The peripheral wells were loaded with sera obtained from monkey, rat, cat, dog, rabbit and human. The plates were incubated in a moist chamber at room temperature and were observed for the precipitin lines after 24 hrs and 48 hrs.

Among others only monkey serum was used at different dilutions (neat, 1:2, 1:4, 1:8) to check cross reactivity with anti-human C_3 by Ouchterlony technique as described above.

For immunofluorescence studies fresh frozen sections of monkey liver were incubated with fluorescein tagged rabbit anti-human C_3 (dilutions 1:5 and 1:10) for 30 min. at 37° C and then washed thoroughly in PBS. For control, other tissue such as pituitary was processed exactly as liver sections. Preparations were studied under Carl Zeiss universal microscope with 200 lamp and excitation filters of KP 500 and a barrier filter of 55.

Fresh frozen sections of monkey liver were incubated in anti-C_3 component of human, raised in rabbits, for half an hour to an hour at 37° C for sandwich technique using peroxidase as probe. After thorough washing in phosphate buffer saline (PBS) the sections were treated with sheep anti-rabbit immunoglobulin tagged with peroxidase (IgG–HRP) for 1/2 hr at 37° C. Sections were washed three times in excess of PBS. Peroxidase was localized by incubating the sections with 2 mg/10 ml diaminobenzidine (DAB) in 0·05 M Tris-HCl buffer pH 7·5 containing a drop of H_2O_2 for 10 min in dark at room temperature[5,6] and slides were then washed in tris buffer, dehydrated

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