will elicit a neural response in the epiblast[87,102–106]. Under certain conditions, the epiblast seems to be neuralized even without any specific inductor[107,108]. In all these respects, the avian epiblast resembles Amphibian ectoderm; evidently the neural tendencies are inherent in the cells, and maybe only some metabolic block has to be removed at a proper time in order to start the neuralization.

The biochemical nature of this neuralization is practically unknown. A treatment of the node with pyridine[109] or colchicine[110] will destroy the inductive power of the node, whereas actinomycin D, which blocks the synthesis of messenger RNA, does not affect it[111]. But we do not even know whether these facts are relevant to the actual biochemistry of the induction and determination.

Conclusion

If the hundred years of study on the HENSEN's node – i.e. on gastrulation and early determination of the embryos of amniote vertebrates – teach anything, they teach in the first place how limited and fragmentary our knowledge is about one of the most central problems of the whole developmental biology. We know that the events in early amniote development – or early avian development, on which our data and ideas are nearly all based – in many ways resemble those in early Amphibian development, which is only slightly better understood, but we also know that direct extrapolations from anamniotes to amniotes cannot be made without proper reservations and without studying the amniote embryos themselves. And we have practically no idea of what is really going on in the cells of the blastoderm when they move, invaginate, induce or are induced, interact, become determined and begin their differentiation. We know that at the stages of gastrulation, the node, and indeed the whole blastoderm, is in a very labile state and can be regulated in many ways to produce a harmonious whole – or a monster – although we only understand very poorly the modes of this regulation. The progress made during the decades, and particularly in recent years, shows, however, that useful information is accumulating to produce a coherent picture, and there is no reason to be pessimistic[112].

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SPECIALIA

Novel Reactions of Rotenone

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Summary. Novel reactions of rotenone are described. Demethylation of rotenone under mild conditions was observed. A compound with oxidation state between rotenone and rotenonone was isolated.

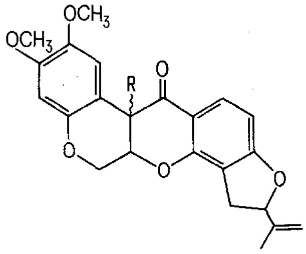
A programme of work aimed at blocking in vivo hydroxylation sites of rotenone and examining reactions of the carbonyl group has led to the synthesis of some novel rotenoids.

Material and methods. Action of cupric nitrate on rotenone. Cupric nitrate (400 mg) was added in portions to rotenone (1.3 g) in acetic anhydride (25 ml). After 18 h the mixture was poured on to ice. The gum which separated was extracted with chloroform and chromatographed on alumina in chloroform. Evaporation of chloroform eluates gave yellow solid (160 mg, 12%) **4** mp. p 243–245° (petrol/ethyl acetate) $C_{22}H_{18}O_7$ ($CDCl_3$/$DMSOd_6$): 3.86 (3H, s, 2–OCH_3); 6.07 (1H, d, J6.0, 6–CH); 6.61 (1H, s, 4-ArH); 7.62 (1H, d, J6.0, 6–OH); 8.42 (1H, s, 1-ArH) m/e 394 (M^+ 394).

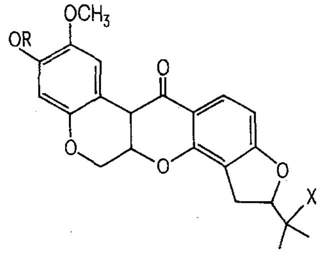
Action of hydrogen bromide on rotenone. Rotenone (2.0 g) in acetic acid (100 ml) was saturated at 0° over 2 h. After 72 h, the mixture was poured into water, the solid collected and chromatographed on alumina in chloroform. Colourless solid (750 mg, 29%) **7** was obtained by evaporation of the eluates m.p. 145–147° (methanol) $C_{24}H_2O_3Br_7$ γ_{max} (Nujol) 1680 (CO), 1760 ($COCH_3$) cm^{-1}. ($CDCl_3$) 2.20 (3H, s, $OCOCH_3$); 3.70 (3H, s, 2-OCH_3); 6.46 (H, s, 4-ArH); 6.92 (1H, s, 1-ArH), *m/e* 503 (M^+ 503).

Action of cupric bromide on rotenone. Rotenone (1.3 g) and cupric bromide (400 mg) were heated under reflux for 5 h in chloroform (10 ml) and ethyl acetate (10 ml). The solvent was decanted from copper residues and chromatographed on alumina in chloroform to give yellow dehydrorotenone (500 mg; 28%) 228–229°.

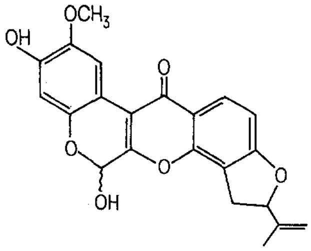
Action of methyl magnesium iodide on rotenone. Rotenone (1.3 g) was added to methyl magnesium iodide (from magnesium 100 mg and methyl iodide 500 mg) in tetrahydrofuran (10 ml) and the mixture stirred for 18 h. The mixture was decomposed with ammonium chloride solution and extracted with chloroform. Chromatography on alumina in chloroform afforded (300 mg; 22%) m.p. 87–88° of 6aβ 12aβ rotenolone.



(1) R = H, 5ß
(2) R = Br
(3) R = CH_3



(5) R = CH_3, X = Cl
(6) R = CH_3, X = Br
(7) R = $COCH_3$, X = Br



(4)

Action of methyl iodide on rotenone. Rotenone (1.3 g) was added to sodium hydride (150 mg; 60% dispersion) in dimethylformanide (15 ml). Methyl iodide (2.5 ml) was added and the mixture heated reflux for 5 h. The solvent was evaporated and the residue chromatographed on alumina in petrol/chloroform mixtures 1:1 to give colourless solid (330 mg, 24%) **3** m.p. 68–70° $C_{24}H_{24}O_6$ ($CDCl_3$) 1.49 (3H, s, 12aCH_3); 3.79 (3H, s, 2-OCH_3); 3.90 (3H, s, 3-OCH_3); 4.40 (1H, m, 6a-H); 4.40 (1H, m, 6-H); 4.70 (1H, d,d, J10.0, J5.0, 6-H); 6.36 (1H, s, 4-ArH); 7.70 (1H, s, 1-ArH), *m/e* 408, (M^+ 408).

Action of ethyl chloroformate on rotenone oxime. The oxime (1.0 g), pyridine (0.2 ml) and ethyl chloroformate (0.3 ml) were stirred at room temperature for 3 h in methylene chloride (15 ml). The solvent was evaporated and the residue crystallized from aqueous ethanol to give the colourless ester (1.2 g; 100%) m.p. 228–230° $C_{26}H_{27}O_8$ γ_{max} (Nujol) 1780 ($CO_2C_2H_5$) cm^{-1}.

Action of acetic anhydride on rotenone oxime. The oxime (1.0 g) was heated in acetic anhydride (10 ml) at 100° for 5 h and the mixture poured into water. Crystallization of the colourless product (600 mg; 53%) was from aqueous alcohol mp. 202–204° $C_{25}H_{25}O_7N$ γ_{max} (Nujol) 1750 ($COCH_3$) cm^{-1}.

Chromatography alumina was Woelm Grade 3 and rotenone was used as supplied by the Aldrich Chemical Co. Ltd.

Results and Discussion. Rotenone **1** was allowed to react with cupric nitrate in acetic anhydride at room temperature. Dehydrogenation of the B/C ring junction and partial oxidation of the 6-methylene group accompanied demethylation to give **4**. This compound has a novel oxidation level for the B and C rings between that of rotenone and rotenonone. Although rotenone **1** reacted with hydrogen chloride in glacial acetic acid at room temperature to give the known[1] addition product **3** the analogous bromo compound **6** could not be obtained. Hydrobromination of the side chain occurred together with demethylation and acetylation of the 3-methoxyl group to give **7**. An attempt to dehydrobrominate **7** with ethanolic silver acetate solution afforded a complex mixture of products which could not be separated but the ^1H NMR-spectrum indicated that some of the desired product was present. Treatment of rotenone **1** with cupric bromide in boiling chloroform/ethyl acetate 1:1 failed to give the desired α bromoketone **2** and the known dehydrorotenone was isolated. As hydrogen bromide was evolved during the reaction bromination was presumably followed by dehydrobromination. The immediate reaction which occurred when methyl magnesium iodide was added to rotenone in tetrahydrofuran at room temperature was base catalyzed incorporation of oxygen to the known[2] solvated 6aβ, 12aβ, $5^1\beta$-rotenolone. A reaction carried out under nitrogen gave only unchanged rotenone on decomposition of the Grignard reaction mixture. Methylation of the enolate anion of rotenone with methyl sulphate in the presence of potassium carbonate gives the known methyl rotenone[3] but with methyl iodide and sodium hydride in dimethylformamide reaction occurs at the softer carbon atom to give the 12a substituted **3**. The ^1H NMR-spectrum shows it is a *trans*-isomer as the chemical shift for the 1-poton indicates shielding by the 12-carbonyl group[4]. Conventional reaction of rotenone oxime with ethyl chloroformate at room temperature and acetic anhydride at 100° gave high yields of the respective esters. All the compounds described had δ and J values in their ^1H NMR-spectra similar to those for rotenone and its derivatives. The mass spectral fragmentation patterns supported the assigned structures of the A and B rings[5] and the elemental analyses of the new compounds were satisfactory.

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