Synthesis of 2-Nitrosofluorene by Peracetic Acid Oxidation of 2-Aminofluorene

In 1961 we reported the *N*-hydroxylation of 2-aminofluorene by rat liver microsomes[1]. The *N*-hydroxylation products were extracted in the form of nitrosofluorene into CCl_4 after oxidizing the diluted incubation mixtures with ferricyanide. The reference compound 2-nitrosofluorene was prepared by the peracetic acid oxidation of 2-aminofluorene according to the method of HOLMES and BAYER[2].

GUTMANN[3] in 1964 stated that when the peracetic acid oxidation is applied to 2-aminofluorene the principal products of the reaction are 2,2'-azoxybisfluorene and 2-nitrofluorene. MILLER and co-workers[4] also emphasized that they likewise failed in attempts to prepare 2-nitrosofluorene by this method.

From these data KIESE[5] adduced that we therefore have only tried to find the microsomal *N*-hydroxylation of 2-aminofluorene and that we failed to substantiate our claims to have synthetized 2-nitrosofluorene and to have detected it in suspensions of microsomes incubated with 2-aminofluorene.

Therefore, we feel that a detailed description of the synthesis is appropriate.

One gram of 2-aminofluorene (5.52 mmoles, F = 129 to 130 °C) is dissolved in 20 ml of glacial acetic acid. 6.0 ml of 30% aqueous H_2O_2 (Merck, pro analysi) are added, the mixture is warmed to 45–50 °C for 3 min and is then allowed to come to room temperature. Direct light should be excluded. After several minutes the light yellow solution turns emerald green.

The formation of 2-nitrosofluorene was checked by thin layer chromatography. Three μl of the reaction mixtures were applied to plates coated with silica gel HF_{254} (Merck, Darmstadt); development with petrol ether (40–60 °C) plus acetone; 4 + 1. The spots of nitrosofluorene, Rf 0.7, were detected by UV-absorption and by spraying with 5% aqueous trisodium pentacyanoamine ferrate. This procedure was also found to be useful to follow the appearance of nitroso or *N*-hydroxy derivatives during synthetic oxidation of arylamines or during reduction of nitro compounds.

Two hundred ml of cold water are added after 30 min and the mixture is extracted twice with 40 ml portions of CCl_4. The aqueous phase is then treated with 10 ml of a 5% solution of ferriammoniumsulphate and is extracted another 2 times with 30 ml portions of CCl_4. The combined CCl_4 extracts are washed once with equal volumes of 1 *N* H_2SO_4 and twice with cold water.

The CCl_4 is dried over Na_2SO_4 for 30 min and then is evaporated in vacuo at 20–25 °C. The yellow brown residue is dissolved in a minimum of warm *n*-hexane and placed on a 20 × 200 mm column of silica gel Woelm (M. Woelm, Eschwege). The column is developed with *n*-hexane. A deep green band of 2-nitrosofluorene moves in front of the other coloured substances. The green band is collected and evaporated to dryness at 20–25 °C in vacuo yielding emerald green crystals, F = 76–77 °C. The yield of several experiments was 6–15%. The UV-absorption spectra was identical with that of 2-nitrosofluorene synthetized by reduction of 2-nitrofluorene and consequent oxidation of the hydroxylamine as described by LOTLIKAR et al.[4]. The mixture melting point gave no depression.

The direct oxidation of several arylamines according to HOLMES and BAYER[2] and purification by means of column chromatography for the synthesis of nitroso derivatives offers certain advantages compared with other known methods.

Zusammenfassung. Lösungen von 2-Aminofluoren in Eisessig färben sich nach Zugabe von H_2O_2 bald tiefgrün. Das bei der Oxydation mit Peressigsäure entstehende 2-Nitrosofluoren lässt sich leicht extrahieren und nach Säulentrennung rein gewinnen. Dieses Verfahren ist einfacher und schneller als andere bekannte Methoden.

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The Synthesis of Jaceidin

Jacein (I) has been isolated from the leaves and stems of *Centaurea jacea* L. by L. FARKAS et al.[1]. Hydrolysis of I with hydrochloric acid afforded an aglycone, jaceidin (II). They established the aglycone (II) as 5,7,4'-trihydroxy-3,6,3'-trimethoxyflavone on the basis of analytical and degradative studies. Recently, I and II have been isolated from the *Centaurea* species by J. H. BOWIE et al.[2] and F. BOHLMANN et al.[3]. In this paper, we wish to report the total synthesis of II confirming the proposed structure.

The Hoesch reaction of iretol with methoxyacetonitrile yielded 2,4,6-trihydroxy-3,ω-dimethoxyacetophenone (III) (m.p. 157–158 °C. Found: C, 52.94; H, 5.31. $C_{10}H_{12}O_6$ requires: C, 52.63; H, 5.30%). According to ALLAN-ROBINSON's flavone synthesis, the condensation of III with *O*-benzylvanillic anhydride (IV) in the presence of triethylamine, followed by treatment with alcoholic potassium hydroxide, afforded a hydroxyflavone (m.p. 181.5–183.0 °C, IR 3360 (OH), 1655 cm^{-1} (γ-pyrone) (Nujol), UV λ_{max}^{EtOH} nm ($\log\varepsilon$); 256 (4.21), 274 (4.20), 347 (4.31). Found: C, 66.86; H, 5.06. $C_{25}H_{22}O_8$ requires: C, 66.66; H, 4.92%). Two structures (V and VI) were expected for this flavone. In order to elucidate the structure of the flavone, a diethyl derivative, which was easily

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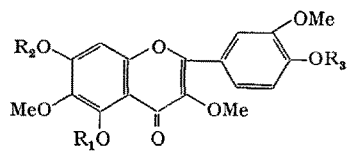
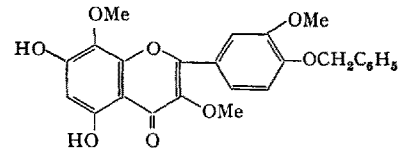
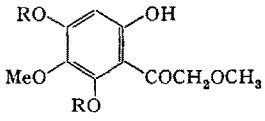


I $R_1 = R_3 = H$, R_2 = Glycosyl
II $R_1 = R_2 = R_3 = H$
V $R_1 = R_2 = H$, $R_3 = CH_2C_6H_5$
VIII $R_1 = R_2 = Et$, $R_3 = CH_2C_6H_5$
IX $R_1 = R_2 = R_3 = CH_3CO$
X $R_1 = R_2 = R_3 = Me$
XI $R_1 = R_2 = R_3 = Et$



VI



III R = H
VII R = Et

obtained with diethyl sulphate, was prepared from 3,5-diethoxy-4-methoxyphenol by an unambiguous method.

By a similar Hoesch condensation the phenol gave 4,6-diethoxy-2-hydroxy-5,ω-dimethoxyacetophenone (VII) (b.p. 145–146 °C/0.2 mm. Found: C, 59.24; H, 7.37. $C_{14}H_{20}O_6$ requires: C, 59.14; H, 7.09%). According to the ALLAN-ROBINSON's flavone synthesis, the ketone (VII) with IV yielded 4'-benzyloxy-5,7-diethoxy-3,6,3'-trimethoxyflavone (VIII) (m.p. 117–118 °C, IR 1638 cm^{-1} (γ-pyrone) (Nujol), UV λ_{max}^{EtOH} nm (log ε): 336 (4.27). Found: C, 69.03; H, 6.04. $C_{29}H_{30}O_8$ requires: C, 68.76; H, 5.97%). This compound was identified by direct comparison with the diethyl compound, which was obtained by the ethylation of the hydroxyflavone. From this fact, the structure of the hydroxyflavone was established as 4'-benzyloxy-5,7-dihydroxy-3,6,3'-trimethoxyflavone (V). Then, the debenzylation of V with hydrogen afforded the desired flavone (II) (m.p. 130–135 °C (from methanol-water). Found: C, 58.57; H, 4.82. $C_{18}H_{16}O_8 \cdot {}^1/_2H_2O$ requires: C, 58.54; H, 4.64%. After drying 50–60 °C/10^{-1} mm for 2 h, m.p. 166.0–166.5 °C (127 °C sinter). Found: C, 59.86; H, 4.48. $C_{18}H_{16}O_8$ requires: C, 60.00; H, 4.48%. IR 3560, 3250 (OH), 1655 cm^{-1} (γ-pyrone) (Nujol), UV λ_{max}^{EtOH} nm (log ε): 257 (4.18), 272.5 (4.20), 355 (4.28) (lit.[1] m.p. 127–133 °C, UV λ_{max}^{MeOH} nm (log ε): 255 (4.23), 350 (4.32) (lit.[2] m.p. 165–166 °C, UV λ_{max}^{EtOH} nm (log ε): 255 (4.25), 270 (4.19), 354 (4.35), whose identity with natural jaceidin[4] was confirmed by mixed m.p. determination, IR- and UV-spectral comparison. Its triacetate (IX) (m.p. 163–164 °C) (lit.[2] m.p. 159–160 °C), trimethyl ether (X) (m.p. 142–143 °C) (lit.[2] m.p. 142–143 °C) and triethyl ether (XI) (m.p. 118–119 °C (lit.[1] m.p. 118 °C) were prepared by a usual method. Also, XI could be derived from VII and *O*-ethylvanillic anhydride by a similar method.

Zusammenfassung. Durch Kondensation nach ALLAN-ROBINSON wurde aus 2,4,6-Trihydroxy-3,ω-dimethoxyacetophenon das 4'-Benzyloxy-5,7-dihydroxy-3,6,3'-trimethoxyflavon dargestellt. Entbenzylierung in C-4'-Stellung führte zum 5,7,4'-Trihydroxy-3,6,3'-trimethoxyflavon. Die Eigenschaften dieser Verbindung und ihre Derivate sind identisch mit dem aus *Centaurea jacea* L. isolierten Jaceidin bzw. seinen Derivaten.

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[4] We are grateful to Prof. H. WAGNER, University of München, for his gifts of natural jacein and jaceidin.

The Chemistry of Thespesin[1]

Thespesin[2], a yellow crystalline compound, m.p. 196 to 197 °C, $[\alpha]_D$ + 457° (benzene), isolated from the fruit of *Thespesia populens* Soland, has now been assigned the molecular formula $C_{30}H_{30}O_8$ on the basis of its mass spectrum (M^+ 518).

Three interesting features of the chemistry of thespesin are the presence of potential aldehyde functions in the molecule, the apparent symmetry of the molecule, and its strong dextrorotation. The IR-spectrum of thespesin is devoid of any C=O absorption between 1625 and 1800 cm^{-1}, but thespesin readily forms a dioxime m.p. 320 °C, a dianilino derivative m.p. 305 °C and a di-2,4-dinitrophenyl hydrazone m.p. 215–220 °C. On methylation in acetone with dimethyl sulphate in the presence of potassium carbonate, a colourless hexamethyl ether $C_{36}H_{42}O_8$ (M^+ 602), m.p. 242–244 °C, $[\alpha]_D$ + 177° (chloroform) is formed. This compound has a discernible aldehyde carbonyl function (ν_{max}^{KBr} 1690 cm^{-1}) and forms the expected dioxime m.p. 200–204 °C and a diphenyl hydrazone m.p. 266–268 °C. These observations can be explained if the thespesin molecule has 2 hemiacetyl functions.

The apparent symmetry of the thespesin molecule is indicated by the uniqueness of the NMR-spectrum of

[1] Communication No. 1225 from the Central Drug Research Institute.

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