

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

In 1961 we reported the *N*-hydroxylation of 2-aminofluorene by rat liver microsomes¹. The *N*-hydroxylation products were extracted in the form of nitrosofluorene into CCl₄ 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 BAYER².

GUTMANN³ 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-workers⁴ also emphasized that they likewise failed in attempts to prepare 2-nitrosofluorene by this method.

From these data KIESE⁵ 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 synthesized 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 H₂O₂ (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 μ l of the reaction mixtures were applied to plates coated with silica gel HF₂₅₄ (Merck, Darmstadt); development with petrol ether (40–60°C) plus acetone; 4 + 1. The spots of nitrosofluorene, *R_f* 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 CCl₄. 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 CCl₄. The combined

CCl₄ extracts are washed once with equal volumes of 1*N* H₂SO₄ and twice with cold water.

The CCl₄ is dried over Na₂SO₄ 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 synthesized 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 BAYER² 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 H₂O₂ 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.

H. UEHLEKE

*Institute of Pharmacology, University of Tübingen
74 Tübingen (Germany), 29 November 1967.*

¹ H. UEHLEKE, *Experientia* 17, 557 (1961).

² R. R. HOLMES and R. P. A. BAYER, *J. Am. chem. Soc.* 82, 3454 (1960).

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⁴ D. P. LOTLIKAR, E. C. MILLER, J. A. MILLER and A. MARGRETH, *Cancer Res.* 25, 1743 (1965).

⁵ M. KIESE, G. RENNER und I. WIEDEMANN, *Arch. exp. Path. Pharmac.* 252, 418 (1966).

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. C₁₀H₁₂O₈ 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 ϵ): 256 (4.21), 274 (4.20), 347 (4.31). Found: C, 66.86; H, 5.06. C₂₅H₂₂O₈ 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

¹ L. FARKAS, L. HÖRHAMMER, H. WAGNER, H. RÖSLER and R. GURNIAK, *Chem. Ber.* 97, 610 (1964).

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³ F. BOHLMANN and C. ZDERO, *Tetrahedron Lett.* 33, 3239 (1967).