Photooxidation of Reserpine

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Abstract □ The final product of the irradiation of reserpine in a chloroform solution was isolated and shown to be 3,4,5,6-tetradehydroreserpine. The structure was determined by NMR spectroscopy as well as by chemical oxidation of reserpine. 3,4-Dehydroreserpine was found to be an intermediate in both the chemical and photooxidation reactions. TLC examination of several reserpine tablets showed the presence of small amounts of dehydroreserpine but no tetradehydroreserpine.

Keyphrases □ Reserpine—photooxidation products, isolation, identification of 3,4,5,6-tetradehydroreserpine □ TLC—isolation of reserpine photooxidation products □ 3,4,5,6-Tetradehydroreserpine—isolation, identification as final product of reserpine photooxidation □ Photooxidation products, reserpine—isolation, identification from chloroform solutions □ NMR spectroscopy—identification of reserpine photooxidation products

It is well known (1, 2) that reserpine (I) is unstable to heat and light and in chloroform solutions. Irradiation of reserpine solutions in both chloroform and methanol was reported (3) to cause darkening of the solutions with the appearance of blue fluorescence. Paper chromatographic analysis (3) of the photolysis reaction demonstrated the appearance of 3-isoreserpine and 3,4-dehydroreserpine (II) as well as a bright-blue fluorescent compound named "lumireserpine." Lumireserpine was the final product upon prolonged irradiation both in chloroform and methanol. It was reported (3) to show neither hypotensive activity nor an increase in epinephrine activity on blood pressure in cats. Presumably only the reserpic acid moiety is affected by irradiation since rescinnamine and deserpidine were reported (3) to undergo a similar decomposition.

In a study related to biosynthesis of reserpine, small amounts of the alkaloid were isolated from *Rauwolfia canescens* by preparative TLC. Chromatography (silica gel G) of nominally pure reserpine (both commercially obtained and isolated from plants) consistently showed several additional compounds (detected by UV lamp) on the TLC plates. Reserpine isolated from the adsorbent with acetone–chloroform (7:3) always showed the additional spots upon further TLC, including the blue fluorescent component suggestive of lumireserpine. It was thought necessary to determine the structure of this product.

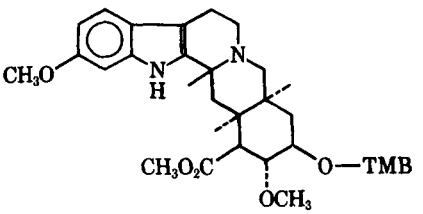
EXPERIMENTAL[1]

TLC—Silica gel G[2] glass plates were prepared with a spreader[3], maintaining adsorbent thicknesses at 250 μ. The coated plates were dried at 110° for 30 min. and then stored in a desiccator. Elution solvents were certified grade.

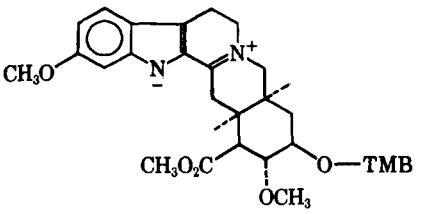
Irradiation of Reserpine—A solution of 1 g. of reserpine in 200 ml. of chloroform in a flask (Vycor) was irradiated with a mercury arc lamp[4]. The reaction mixture was checked periodically by TLC (chloroform–methanol, 4:1). After 120 hr. of irradiation, the intensity of the blue fluorescent spot on the plates corresponding to lumireserpine (R_f 0.45) reached a maximum. The reaction was stopped, and evaporation of the solvent gave a gummy residue. The residue was passed through a silica gel G column (45 g.); 0.25 g. of brown solid, m.p.[5] 170–175° dec., was obtained by elution with chloroform–methanol (4:1). Lumireserpine dissolves in methanol, ethanol, acetone, chloroform, and hot concentrated hydrochloric acid; UV(methanol): $\lambda_{max.}$ 266 and 345 nm.; IR(KBr): 3.40, 3.51 (CH stretching), 5.82 (C=O stretching), and 6.12 μ (C=N stretching); NMR (dimethyl sulfoxide-d_6) δ: 8.43 (doublet, J = 7 Hz., H-5), 8.23 (doublet, J = 7 Hz., H-6), 7.96 (doublet, J = 8 Hz., H-9), 7.40 (doublet, J = 3 Hz., H-12), 7.30 (singlet, 3,4,5-trimethoxybenzoyl ring protons), 7.00 (broad doublet, J = 8 Hz., H-10), 3.99 (singlet, six methoxy protons), 3.88 (singlet, nine methoxy protons), and 3.55 (singlet, three methoxy protons).

The perchlorate salt was prepared by dissolving a small amount of lumireserpine in hot water and adding 70% perchloric acid. Upon cooling, the resulting precipitate was filtered and crystallized from methanol–isopropyl alcohol, m.p. 180–185° dec. [lit. (4) m.p. 194–196° for tetradehydroreserpine perchlorate); UV (methanol): $\lambda_{max.}$ 265, 293, and 373 nm. [lit. (4) UV (ethanol): $\lambda_{max.}$ 265, 295, and 382 nm.].

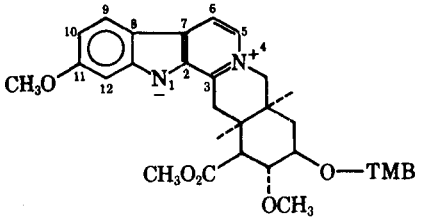
Oxidation of Reserpine with Lead Tetraacetate—*Method A*—To a stirred solution of 0.5 g. of reserpine in 10 ml. of acetic acid was added dropwise 30 ml. of a solution of 1.34 g. lead tetraacetate in acetic acid. After 30 min., most of the acetic acid was removed by



I (TMB = 3,4,5-trimethoxybenzoyl)



II



III

[1] UV and IR spectra were recorded on Perkin-Elmer 350 and 257 spectrophotometers, respectively. NMR spectra were determined on a Jeolco C60-HL spectrometer, external lock mode; chemical shifts are reported in p.p.m. (δ) relative to internal tetramethylsilane.

[2] E. Merck AG, Darmstadt, West Germany.

[3] DESAGA Co., Heidelberg, West Germany.

[4] Hanovia, model SC 5041, 360–370 nm.

[5] Melting points were determined on a Mel-temp apparatus and are uncorrected.

distillation *in vacuo*. Chloroform and a small amount of water were added, and the mixture was made slightly alkaline with 50% aqueous sodium hydroxide. The residue was passed through a neutral alumina column (20 g.). The bright-blue fluorescent product was eluted by acetone–chloroform–methanol (2:1:1). Each fraction collected from the column was examined by TLC. IR, UV, and NMR spectra were also determined; in each case the spectrum was identical to that of lumireserpine.

Method B—The oxidation procedure described for Method A was executed in the same manner through the evaporation step, with the exception that 0.65 g. lead tetraacetate in 20 ml. of acetic acid was employed. TLC examination of the residue showed that the major product was 3,4-dehydroreserpine, with a small amount of lumireserpine being formed. The gummy red residue was passed through a silica gel G column (40 g.), and the apple-green fluorescent dehydroreserpine was eluted with chloroform–methanol (4:1) and finally isolated by preparative TLC, using *n*-hexane–butanone–methanol (3:2:1), R_f 0.5; UV: $\lambda_{max.}$ 260 and 325 nm. It was identical (TLC and UV) to 3,4-dehydroreserpine obtained from the photooxidation reaction.

Reduction of Lumireserpine with Sodium Borohydride—Lumireserpine (100 mg.) was dissolved in 20 ml. of methanol, and 100 mg. of sodium borohydride was added. After refluxing for 20 min., most of the solvent was removed using a vacuum evaporator. The residue was dissolved in chloroform and examined by TLC in solvent systems of *n*-hexane–butanone–methanol (4:4:1) and chloroform–methanol (4:1). Spots corresponding to isoreserpine were detected and were identical (TLC and UV) to an authentic sample. The blue fluorescence of lumireserpine was very weak.

Isoreserpine—Reserpine was isomerized to 3-isoreserpine by refluxing in acetic anhydride for 20 hr. according to MacPhillamy *et al.* (5). The crystallized isoreserpine contained (TLC) small amounts of dehydroreserpine and tetradehydroreserpine. The isoreserpine had m.p. 140–145° [lit. (5) m.p. 150–155°], obtained by preparative TLC in *n*-hexane–butanone–methanol (5:4:1); UV (chloroform): $\lambda_{max.}$ 295 and 266 nm.

Analysis of Reserpine Tablets—Four tablets of each of six commercially available samples were ground to a fine powder with a mortar and pestle and then suspended in 2 ml. of methanol. Each methanolic solution was filtered through a pasteur pipet with glass wool and sea sand, and the filtrate was spotted on glass plates, applying three spots of varying concentrations for each sample. Isoreserpine and dehydroreserpine were also spotted at the same time. The plates were developed with either chloroform–methanol (4:1) or *n*-hexane–butanone–methanol (5:4:1). After development, the plates were air dried. In most cases, trace amounts of isoreserpine and dehydroreserpine were detected. No tetradehydroreserpine was detected in any sample.

RESULTS AND DISCUSSION

Irradiation of dilute solutions of reserpine in chloroform caused eventual darkening of the solutions. The reaction mixture was checked periodically by TLC. Initially, at least three new components were detected. 3-Isoreserpine was detected near the solvent front just above reserpine. The component at R_f 0.65 was found to be 3,4-dehydroreserpine as previously reported (3), with the blue fluorescent compound at R_f 0.45 being the major product after 120 hr. of irradiation. This compound was isolated by column chromatography on silica gel G, and its UV spectrum was identical to that reported for lumireserpine. Sublimation of lumireserpine was attempted, but the slightly-yellow particles collected on the cold finger revealed a number of components (TLC) with no lumireserpine. (The mass spectrum of lumireserpine also indicated it to be thermally unstable.)

The NMR spectrum of lumireserpine in dimethyl sulfoxide-d_6 was similar to that of reserpine with the following differences. The N—H resonance of reserpine at 10.5 p.p.m. (δ) (6) was absent in the spectrum of lumireserpine, as was the 3-H resonance which occurs at 4.45 p.p.m. in reserpine. In addition, two new resonances appeared in the aromatic region of lumireserpine: doublets at 8.23 and 8.34 p.p.m. (J = 7 Hz.). Little else in the spectrum was different from that of reserpine, except for a decrease in the area of the alicyclic resonances. This information suggested that lumireserpine was 3,4,5,6-tetradehydroreserpine (III), an anhydronium base similar to serpentine (hence the blue fluorescence as with serpentine). No attempts were made to exclude air from the reaction mixture; most probably the oxidation involves molecular oxygen as hydrogen acceptor.

Tetradehydroreserpine perchlorate was prepared (4, 7) for stereochemical studies by a maleic acid/palladium black oxidation of either reserpine or isoreserpine. This reaction required 90 hr. to complete. The present authors adapted a literature (5) method for the oxidation of reserpinediol to tetradehydroreserpinediol employing lead tetraacetate in acetic acid, a procedure which is complete in 30 min. The product obtained by the oxidation of reserpine in this way was identical (UV, IR, and NMR) to the photooxidation product lumireserpine, and its perchlorate was identical (UV) to that previously reported for tetradehydroreserpine perchlorate. Furthermore, the use of one-half the amount of oxidizing agent led to the formation of 3,4-dehydroreserpine as the major product. The photolytic as well as the chemical oxidation proceeded through the same intermediate, 3,4-dehydroreserpine. Final evidence for the structure was afforded by the reduction of lumireserpine with sodium borohydride to give 3-isoreserpine, the more stable anomer (8) of reserpine.

Several commercially available brands of reserpine tablets were examined by solvent extraction and TLC for the presence of photooxidation products. In all cases, 3-isoreserpine and 3,4-dehydroreserpine were detected, ranging from slight traces to appreciable (~5%) amounts by visual approximation. No tetradehydroreserpine was detected in any sample.

The conventional methods (9–11) for reserpine assay involve the oxidation of reserpine with nitrous acid, producing dehydroreserpine and tetradehydroreserpine as well as other products, followed by spectrometric, colorimetric, or spectrofluorometric determination of these oxidation products. Banes *et al.* (9) took into account the presence of oxidation products by determining a blank absorbance at 390 nm. prior to nitrous acid oxidation. There was no indication in this study that the amounts of photooxidation products of reserpine exceeded USP limits.

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