# 1-ARYL-3,3-DIALKYLTRIAZENE COMPOUNDS. VI.*

# OXIDATION OF 1-PHENYL-3,3-DIMETHYLTRIAZENE AND OF SOME OF ITS *para*-SUBSTITUTED DERIVATIVES BY MOLECULAR OXYGEN IN UDENFRIED'S MODEL OF HYDROXYLASE

V.ZVĚŘINA, J.DIVIŠ, J.MARHOLD and M.MATRKA

*Research Institute of Organic Syntheses, Pardubice - Rybitví*

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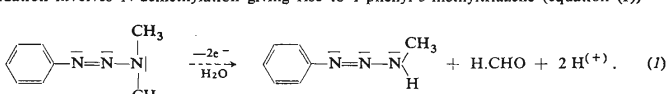
The oxidation of 1-phenyl-3,3-dimethyltriazene and of some of its *para*-substituted derivatives by molecular oxygen in Udenfried's model of hydroxylase was studied. The aim of the present paper was to establish whether the constituent mechanism of the reaction involves also the N-demethylation of the described derivatives, a reaction which takes place during enzymatic oxidation when microsomal fractions of rat liver are used *in vitro*.

1-Aryl-3,3-dimethyltriazene compounds have been studied before and recommended as effective potential carcinostatics[1,2] in view of certain of their biological properties. The effect of these compounds on Friend pernicinous leukemia[3] was investigated. Druckrey and coworkers[4] found recently that even low doses of 1-phenyl-3,3-dimethyltriazene cause the growth of malignant tumors of brain and peripheral nerves in experimental rats. These products have found application so far predominantly as stabilized aromatic diazo compounds in dye industry[5] and as repellents and herbicides[6]. Some problems connected with these compounds have already been studied in our laboratory. We focused our attention on the preparation of certain new derivatives[5] and on the development of analytical methods for their determination[8-18]. Preussmann and his coworkers[17] described recently the enzymatic oxidation of 1-phenyl-3,3-dimethyltriazene and 1-(pyridyl-3)-3,3-dimethyltriazene by microsomal rat liver fractions *in vitro*. They assume that this reaction is related to the mechanism of carcinogenic action of these compounds. This oxidation involves N-demethylation giving rise to 1-phenyl-3-methyltriazene (equation (*I*))

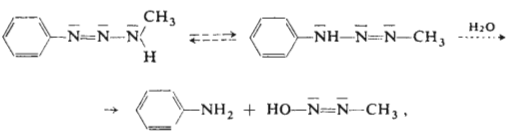



(*I*)

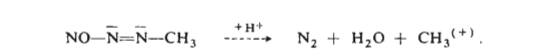
It is assumed that in the first phase of the reaction enzymatic hydroxylation takes place and leads to the formation of a labile intermediary product, 1-phenyl-3-methyl-3-hydroxymethyltriazene

* Preceding papers: I. This Journal *30*, 3959 (1965); II. This Journal *31*, 4735 (1966); III. Chem. listy *61*, 256 (1967); IV. Chem. průmysl *17*, 616 (1967); V. Československ. farm. *18*, 33 (1969).

which is then hydrolytically decomposed to 1-phenyl-3-methyltriazene. The latter affords with water — in accordance with the observations of Dimroth and others[18–20] — aniline and methyldiazo hydroxide which undergoes acid cleavage to the methyl cation, elemental nitrogen, and water (equations (*2*) and (*3*))



(*2*)



(*3*)

From this viewpoint regard Preusmann and v. Hodenberg[20] the temporarily arising 1-aryl-3-methyltriazene as an alkylating agent whose carcinogenic action in the living organism consists in its alkylating attack on biopolymers, predominantly on deoxyribonucleic acids, similarly to the analogous action of other alkylating agents, such as, *e.g.* certain N-nitroso compounds, aliphatic hydrazo-, azo-, and azoxy compounds[21–23]. These authors have proved the correctness of their assumption by the reaction of 1-phenyl-3-methyltriazene with guanosine, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA).

In our laboratory we have studied in addition to the chemical and physical properties of 1-aryl-3,3-dialkyltriazene compounds also their biological characteristics, especially their toxicity[24] and carcinogenic activity[25]. For these reasons we have turned our attention also to their oxidation. In preliminary experiments with electrochemical oxidation of these compounds on a platinum rotatory disc microelectrode we observed that the oxidation does not proceed by direct electron transfer in a medium characterized by the concentration of hydroxonium ions as pH 2 to 10. For these reasons we decided to employ oxidation by molecular oxygen using oxygen transferors of the type of complex iron compounds, in the form of the well-known Udenfriend's model of hydroxylase[26]. The modification used before in studies on the oxidation of another carcinogenic compound, N,N-dimethyl-4-aminoazobenzene[27], was chosen.

Five aryltriazene compounds were subjected to oxidation, *i.e.* 1-phenyl-3,3-dimethyltriazene, 1-(4′-tolyl)-3,3-dimethyltriazene, 1-(4′-methoxyphenyl)-3,3-dimethyltriazene,1-(4′-chlorophenyl)-3,3-dimethyltriazene,and 1-(4′-nitrophenyl)-3,3-dimethyltriazene. We also studied the oxidation of 1-phenyl-3-methyltriazene as an assumed intermediate of the oxidation of 1-phenyl-3,3-dimethyltriazene. The reaction products were identified by thin-layer chromatography on silica gel. Since the course of the oxidation itself was considerably affected by daylight, we carried out our experiments in the dark, at daylight, and eventually, at ultraviolet illumination.

## EXPERIMENTAL

### Products Used

1-Phenyl-3,3-dimethyltriazene, 1-(4′-tolyl)-3,3-dimethyltriazene, 1-(4′-methoxyphenyl)-3,3-dimethyltriazene, 1-(4′-chlorophenyl)-3,3-dimethyltriazene, 1-(4′-nitrophenyl)-3,3-dimethyltriazene, and 1-phenyl-3-methyltriazene were prepared by the procedure described earlier and their purity was checked by thin-layer chromatography on silica gel[18]. The remaining chemicals used, including model amines (aniline, 4-toluidine, 4-anisidine, 4-chloroaniline, and 4-nitraniline) were G. R. and A. G. purity grade products of Lachema and Merck.

### Apparatus and Procedures

The *apparatus* for oxidation by molecular oxygen (Udenfried's model of hydroxylase) has been described in our preceding paper[27]. In experiments where moreover the effect of light was investigated, the reaction was either insulated from daylight by covering the reaction vessel with black paper (the type used for the storage of photographic material) or, on the other hand, a quartz reaction vessel was used and illuminated from the distance of 20 cm by mercury lamp BGW HOA 500 W. The reaction vessel equipped with a dual jacked was temperature-controlled at 40°C by a Höppler ultrathermostat. The oxidations were carried out in the atmosphere of pure medicinal oxygen at a pressure of 760 Torr.

*Procedure*: In the reaction vessel were placed 11 ml of acetone, 23 ml of water, $3 \cdot 10^{-5}$ mol of ethylenediaminetetraacetic acid (sodium salt) dissolved in 7 ml of water, $1{\cdot}5 \cdot 10^{-5}$ mol of ferrous-ammonium sulfate also dissolved in 4 ml of water, $1{\cdot}5 \cdot 10^{-5}$ mol of the corresponding triazene compound dissolved in 4 ml of acetone, $3 \cdot 10^{-4}$ mol of ascorbic acid in 4 ml of water, 0·055 g of potassium primary phosphate and 0·57 g of sodium secondary phosphate (a pH of approximately 7·0 was obtained). After dissolving of all compounds and mechanical stirring, there action solution was thermostated at 40°C. Afterwards the oxidation was carried out under an oxygen pressure of 760 mm with stirring by a vibrator for the required period. After the end of the oxidation the reaction was discontinued by the addition of 0·5% solution of sodium bicarbonate. The reaction mixture was then extracted with 10 ml of chloroform, the chloroform extract was taken to dryness, and the dry residue was dissolved in 1 ml of ethanol. This solution was used directly for chromatographic identification.

*The chromatography* of reaction products was carried out on a thin-layer of silica gel with starch binder, a commercial product (Silufol UV 254, Kavalier) containing a fluorescent indicator. The chromatograms were developed in xylene, the oxidation products of 1-(4′-nitrophenyl)-3,3-dimethyltriazene were separated in benzene. The reaction products were detected in ultraviolet light (ultraviolet lamp "Philora", 500 W, placed at a distance of approximately 30 cm) by the Ehrlich reagent (by this reagent only compounds containing a free amino group in their molecule were detected, see also model amines assumed to be formed by the mechanism given in equation *1* and *2*), and also by spraying the chromatogram with an acid solution of N-phenyl-1-naphthylamine-8-sulfonic acid (2N-HCl in 50% ethanol). The latter reagent detected reliably the presence of unoxidized aryltriazene compounds.

For *electrochemical oxidation* on the platinum rotatory disc microelectrode the apparatus described in one of our preceding studies[29] was used in combination with polarograph LP 60 equipped with electronic recorder EZ 2. The electrochemical oxidation was studied at pH 7·0 in Britton–Robinson[30] buffer containing 50% of acetone. None of the studied derivatives of 1-phenyl-3,3-dimethyltriazene was observed to undergo oxidation. The electrochemical oxidation of 1-phenyl-3-methyltriazene was not carried out.

## RESULTS AND DISCUSSION

While on the platinum disc microelectrode 1-phenyl-3,3-dimethyltriazene and its derivatives did not undergo electrochemical oxidation (which is – according to our preceding experiments with N,N-dimethyl-4-aminobenzene and some of its derivatives – typical of N-demethylation), the course of oxidation by molecular oxygen in Udenfriend's model of hydroxylase was relatively satisfactory. The aim of this study was to determine whether the mechanism of oxidation is identical to that revealed by Preussmann in his experiments with the oxidation of 1-phenyl-3,3-dimethyltriazene by microsomal liver enzymes[17]. For these reasons our attention was focused above all on N-demethylation leading to the formation of the corresponding 1-phenyl-3-methyltriazene (which underwent hydrolysis to the corresponding aniline derivative, *cf.* equations (*1*) and (g). As shown by the data given in Table I, N-demethylation occured only in the case of the fundamental compound, *i.e.* 1-phenyl-3,3-dimethyltriazene, and in the case of 1-(4′-tolyl)-3,3-dimethyltriazene and 1-(4′-chlorophenyl)-3,3-dimethyltriazene. A quantitative comparison of the obtained results shows that the highest quantity of the arising monomethyltriazene (of its hydrolytic product, aniline, respectively) was found in the case of oxidation of the fundamental compound, 1-phenyl-3,3-dimethyltriazene. Among the other oxidation products listed in Table I are also given the $R_F$-values of spots of unoxidized triazene compounds (set in italics). It follows from the remaining data given in the Table that especially 1-phenyl-3,3-dimethyltriazene gives rise to 3 other reaction products whose constitution, similarly to that of the oxidation products of the remaining triazene compounds, has not been determined. A semiquantitative evaluation of the quantity of the corresponding amine arising from the hydrolysis of the primarily formed monomethyltriazene showed that for 1-phenyl-3,3-dimethyltriazene this amount is less than 10%, for 1-(4′-tolyl)-3,3-dimethyltriazene and 1-(4′-chlorophenyl)-3,3-dimethyltriazene less than 5%. This means that, unlike in the case of enzymatic oxidation by microsomal fractions of rat liver (as described in the paper of Preussmann[17]), either the participation of only the N-demethylation mechanism is minimum when Udenfriend's model of hydroxylase is used or the arising 1-phenyl-3-methyltriazene undergoes further oxidation. Moreover, in the case of markedly electronegative substituted derivatives of 1-phenyl-3,3-dimethyltriazene (see nitro derivative), 4-nitraniline was not found at all. A similar observation was made also with 1-(4′-methoxyphenyl)-3,3-dimethyltriazene.

In an effort to show that the monomethyl derivative can be formed as an intermediate which then undergoes further oxidation, we oxidized 1-phenyl-3-methyltriazene, the primary product of oxidative N-demethylation. We found, that during the oxidation the product is completely eliminated and that the oxidation products are approximately identical with the oxidation products of the starting 1-phenyl-3,3-dimethyltriazene. In addition to the spots characterized by their $R_F$-values in Table I, two additional spots of $R_F$ 0·057 and 0·19 were found. Aniline was detected

TABLE I

Oxidation Products of 1-Phenyl-3,3-dimethyltriazene and of Some of its Derivatives by Molecular Oxygen in Udenfriend's Model of Hydroxylase in Absence of Light, Identified by Thin-Layer Chromatography on Silica Gel

| Compound | 4-Substituted aniline[a] derivative | | $R_F$ of additional products[b] | Time of oxidation |
|---|---|---|---|---|
| | Quantity | $R_F$ | | |
| 1-Phenyl-3,3-dimethyltriazine | ++ | (0·182) | 0·045; 0·082; 0·272; | 5 hours |
| 1-(4′-Tolyl)-3,3-dimethylthiazene | ++ | (0,073) | 0·045; *0·31* | 4 hours |
| 1-(4-Nitrophenyl)-3,3-dimethyl-triazene | 0 | (0·081) | 0·07; 0·18; *0·36*; | 4 hours |
| 1-(4′-Chlorophenyl)-3,3-dimethyl-triazene | + | (0·155) | 0·07; 0·18; *0·40* | 5 hours |
| 1-(4′-Methoxyphenyl)-3,3-dimethyl-triazene | 0 | (0·045) | 0·08; *0·17*; 0·83 | 5 hours |

[a] Formed by decomposition of intermediarily formed *p*-substituted 1-aryl-3-monomethyltriazene [b] the value for unreacted aryltriazene is set in italics.

among the oxidation products. By this approach we were able to show that the oxidation by molecular oxygen in Udenfriend's model of hydroxylase is more progressive than the enzymatic oxidation by microsomal liver fractions, studied by Preussmann[17], and does not stop at the stage of formation of 1-phenyl-3-methyltriazene or of the corresponding amine, respectively.

Another fact deserving interest was that the oxidation is markedly affected by ultraviolet light. When the reaction vessel was illuminated by a mercury lamp the reaction rate was not only several times higher (approximately 3–4 times) but the pattern of the reaction products was also more complex. Thus, *e.g.* the fundamental compound, 1-phenyl-3,3-dimethyltriazene afforded additional products of $R_F$-values 0·091, 0·236, 0·31, 0·56, 0·675. By contrast, the products of $R_F$-values 0·045, 0·082, and 0·272 were not detected. It would thus appear that the effect of ultraviolet light is connected with increased reactivity which has been observed with the coupling reactions of 1-aryl-3,3-dialkyltriazene compounds in anhydrous media by LeFévre and Liddiocet[31]. These authors explained this phenomenon by the formation of the more active *cis*-isomer.

We did not identify the individual products. We assume though that in most cases we were dealing with hydroxylated derivatives in the aromatic ring, as indicated not only by the fluorescence of the reaction products but also by the fact that the C-hydro-

xylation of aromatic compounds by molecular oxygen is preferred in Udenfriend's model.

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REFERENCES

1. Clarke D. A., Barclay R. K., Stock C. Ch., Rondesvedt Cl. S., Müller J. A., Pagliaro A., O'Longlin J., Hogan P.: Proc. Soc. Exptl. Biol. Med. *90*, 489 (1950).
2. Dogg C. P., Karnovsky D. A., Stock C. Ch.: Proc. Soc. Exptl. Biol. Med. *90*, 489 (1955).
3. Kanemetsu Sugiura, Stock Ch. C.: Acta Unio. Intern. contra Cancrum *16*, 780 (1960).
4. Druckrey H., Ivankovic S., Pressmann R.: Naturwissenschaften *54*, 171 (1967).
5. Ullmann F.: *Enzyklopädie der Technischen Chemie*, Part IV, p. 141. Urban & Schwarzenberg, Munich–Berlin 1953.
6. US-Pat. 3 206 357 (1965).
7. Zvěřina V., Diviš J., Marhold J., Matrka M.: Českoslov. farm. *18*, 33 (1969).
8. Lohr L. J.: Anal. Chem. *25*, 1117 (1953).
9. Zvěřina V., Ságner Z., Chmátal V., Matrka M.: Chem. průmysl, *20*, 330 (1970).
10. Matrka M., Marhold J., Ságner Z., Štěrba V.: This Journal *30*, 3959 (1965).
11. Matrka M., Marhold J., Pipalová J.: This Journal *31*, 4735 (1966).
12. Matrka M., Marhold J.: This Journal *31*, 4178 (1966).
13. Matrka M., Chmátal V., Zvěřina V.: Chem. listy *61*, 256 (1967).
14. Zvěřina V., Diviš J., Marhold J., Matrka M.: Chem. průmysl *17*, 616 (1967).
15. Matrka M., Zvěřina V., Ságner Z., Marhold J.: This Journal *34*, 1413 (1969).
16. Zvěřina V., Chmátal V., Junková I., Diviš J., Matrka M.: Chem. průmysl *20*, 231 (1970).
17. Preussmann R., v. Hodenberg A., Hengy H.: Biochem. Pharmacol. *18*, 1 (1969).
18. Dimroth O.: Ber. *36*, 909 (1903).
19. Dimroth O.: Ber. *38*, 670 (1905).
20. Preussmann R., v. Hodenberg A.: Biochem. Pharmacol. in press.
21. Badger G. M., Teubner K. J.: Australian J. Sci. *30*, 239 (1968).
22. Kriek E., Emmelot P.: Biochem. Biophys. Acta *91*, 59 (1964).
23. Byvoet P., Busch H.: Cancer Res. *22*, 249 (1962).
24. Andrýsová O., Rambousek V., Zvěřina V., Matrka M., Marhold J.: Physiol. Bohemoslov., in press.
25. Rambousek V., Jirásek J., Zvěřina V., Matrka M., Marhold J.: Unpublished results.
26. Udenfried S., Clark C. T., Axelrod J. U., Brodis B. B.: J. Biol. Chem. *208*, 731 (1954).
27. Matrka M., Marhold J., Ságner Z., Pipalová J.: This Journal *33*, 3761 (1968).
28. Zvěřina V., Diviš J., Matrka M.: This Journal, in press.
29. Matrka M., Marhold J., Ságner Z.: This Journal *34*, 1615 (1969).
30. Matrka M., Pipalová J., Ságner Z., Marhold J.: Chem. průmysl, *21*, 14 (1971).
31. LeFévre R. W. J., Liddiocet T. H.: J. Chem. Soc. *1951*, 2743.

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