Results and Discussion

The oxygen contents measured by the four methods are summarized in Table I.

The results with oxygen and air saturated water samples demonstrate that the Lex-O_2 method is precise and compares well with Clark-type electrode measurements. Because of the higher viscosities and more complicated handling the other samples show increased standard deviations.

In comparison with the other methods, the Lex-O_2 assay appears to provide realistic results under various conditions. The results with the Clark-type electrodes cannot be directly compared in each case, because these electrodes work only in aqueous systems. In principle the GC-headspace method is also suitable to determine oxygen contents in lipophilic substances or in heterogeneous systems. The precision of this method is dependent on the detection limits of the catharometer, which is in the same range as that of the Lex-O_2. The results are very similar to those obtained with the Lex-O_2, but the standard deviations are often considerably higher. This is certainly due to the more complicated procedure, e.g. the need for absolute exclusion of ambient air by rinsing with helium, and the transfer of the oxygen-saturated samples by means of a syringe into the oxygen-free and sealed test tubes. Even most careful processing during these manipulations cannot prevent certain losses of oxygen, as the samples cannot be applied directly. Another disadvantage is the fact that no internal standard can be used, because each internal standard will get into solubility competition with oxygen. In this case less oxygen would be dissolved.

The oxygen values measured with the van Slyke method also correspond well with those of the Lex-O_2, but this method is extremely time consuming particularly in the case of foaming or highly viscous systems. Another disadvantage is the possibility of water losses during the evacuation phase if there is not sufficient cooling.

Conclusion

The reported results of oxygen content determinations in selected aqueous, oily, and heterogeneous multi-phase systems by means of four different methods demonstrate that the coulometric oxygen assay with Hersch cell detection is simple, fast and accurate. Moreover, it can be applied to non-aqueous systems and complex multi-phase dispersions like emulsions or suspensions.

References

(1) Hersch, P. A. (1960) Anal. Chem. 32, 1030–1034.
(2) Selman, B. J., White, Y.S., Tait, A. R. (1975) Anaesthesia 30, 206–211.
(3) Vennebusch, H., Hellige, G., Prennschütz-Schützenau, H., Sigmund-Duchanova, H., Bretschneider, H. J. (1978) Z. Kardiol. 67, 139–146.
(4) Koch, P., Müller, C. (1974) Prakt. Anästh. 9, 36–39.
(5) Clerbaux, Th., Gerets, G., Frans, A. (1973) J. Lab. Clin. Med. 82, 342–348.
(6) Hitchman, M. L., (1976) Measurement of dissolved Oxygen, (Degn, H., Baslev, I., Brook, R., eds.), pp. 182–184, Elsevier Scientific Publishing Company, Amsterdam-Oxford-New York.
(7) Schuler, P. (1980) Fachzeitschrift für das Laboratorium 24, 299–802.
(8) Leonhardt, A. (1984) Dissertation Freiburg, p. 98.

Chemistry of Primaquine I: Acylation of the Quinoline Ring Portion

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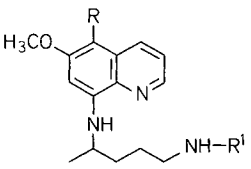
Abstract: Investigation of derivatization of primaquine (**1**) with various perfluoroacylating reagents revealed that the quinoline ring portion of the drug undergoes unexpectedly facile acylation. Chemical and spectral evidence in support of the assigned structures is presented and discussed. Results of the evaluation of these new primaquine derivatives for antimalarial activity is reported.

In the course of an ongoing program to study the chemistry and metabolic fate of primaquine (**1**), a known radical curative agent for relapsing malaria, it was reported that the 5 position of the quinoline ring is unexpectedly reactive (1–3). This high reactivity was indicated by the rapid exchange of H-5 in D_2O (1) and by the formation of an unusual bistrifluoroacetyl derivative (**2**) on treatment of primaquine with excess trifluoroacetic anhydride (2). Finally, the formation of a novel 5-5 methylene linked dimer (3) by certain microorganisms and its ready chemical synthesis provided additional confirmation of this chemical reactivity of the 5 position. Raj Gopalan et al. (4) have reported the formation of an *N,N*-bisheptafluorobutyramide derivative on treatment of primaquine with heptafluorobutric anhydride (HFBA). However, they did not give any spectral evidence in support of their assigned structure. This prompted us to reinvestigate the reaction of primaquine with heptafluorobuytric anhydride and compare the outcome with our results of formation of bistrifluoroacetate **2**. We also wished to prepare sufficient quantities of these materials for biological evaluation as antimalarials.



1 R = R^1 = H
2 R = R^1 = $COCF_3$
3 R = R^1 = $COCF_2CF_2CF_3$
4 R = $COCF_2CF_2CF_3$; R^1 = H
5 R = H ; R^1 = $COCF_2CF_2CF_3$
6 R = $COCF_3$; R^1 = H
7 R = H ; R^1 = $COCF_3$

Materials and Methods

TLC was done on Sil G-25 UV_{254} (Brinkman Instruments, Inc.) plates using A) methanol: chloroform (3:97) and B) (8:92) as solvents. The compounds were

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purified by flash chromatography over Kieselgel 60 (230–400 mesh, Brinkmann Instruments, Inc.) using C) chloroform: hexane (75:25) and D) chloroform. Melting points were determined on a Thomas Hoover apparatus and are uncorrected. IR spectra were run on Perkin-Elmer 281 B spectroplotometer. ^{1}H NMR (90 MHz) was recorded on Varian E-390 spectrometer and ^{13}C NMR spectra (15.03 MHz) were done on a JEOL-FX 60 FT NMR spectrometer using tetramethylsilane as internal standard. MS was recorded on a Finnigan 3200 GC/MS/DS (INCOS) system. Microanalyses were performed on a Hewlett-Packard 185B CHN analyzer at the Departement of Medicinal Chemistry, University of Kansas.

8-(4-Heptafluorobutyrylamido-1-methylbutylamino)-5-heptafluorobutyryl-6-methoxyquinoline (**3**).

The method of Raj Gopalan et al. (4) was used. Primaquine (**1**) free base (0.500 g, 1.53 mmole) and 2 ml of heptafluorobutyric anhydride were dissolved in 20 ml benzene and the solution refluxed for 1.5 h. The reaction mixture was concentrated *in vacuo*, the residue was taken up in chloroform and the chloroform solution washed with saturated sodium bicarbonate, water and dried over anhydrous sodium sulfate. The chloroform was removed *in vacuo* and the crude product was chromatographed over silica gel with hexane and chloroform (1:1) as eluting solvent. The solid obtained recrystallized from benzene-hexane to give bright yellow crystals of **3**, m.p. 98–100°C; 0.9 g (72 %) lit. (4) m.p. 98–100°C: IR (KBr) 1645, 1691; ^{1}H-NMR ($CDCl_3$) δ, 1.37 (d, J=6 Hz, 3H, 1′-CH_3), 1.63–1.83 (bm, 4H, H-2′, 3′), 3.3–3.53 (bt, 2H, H-4′), 3.6–3.93 (bm, 1H, H-1′), 3.97 (s, 3H, OCH_3), 6.23 (s, 1H, H-7), 7.36 (dd, J=4, 8.8 Hz, 1H, H-3), 8.36 (dd, J=1.5, 8.8 Hz, 1H, H-4), 8.5 (dd, J=1.5, 4 Hz, 1H, H-2); ^{13}C-NMR ($CDCl_3$) δ, 145.4 (d, C-2), 124.6 (d, C-3), 132.2 (d, C-4), 128.8 (s, C-4a), 103.5 (s, C-5), 163.8 (s, C-6), 89.4 (d, C-7), 150.6 (s, C-8), 134.1 (s, C-8a), 48.2 (d, C-1′), 33.9 (t, C-2′), 25.9 (t, C-3′), 40.2 (t, C-4′), 20.5 (q, C-5′), 56.1 (q, $ArOCH_3$), 187.4 (t, $ArCOCF_2CF_2CF_3$), 158.2 (t, $NH\text{-}COCF_2CF_2CF_3$); MS; m/z, 651 (M^+ 17 %), 632 (3 %), 482 (M^+–$CH_2CF_2CF_3$, 100 %), 397 (M^+–$(CH_2)_3$ $NHCO_2CF_2CF_3CF$, 10 %), 201 (58 %).

8-(4-Amino-1-methylbutylamino)-5-heptafluorobutyryl-6-methoxyquinoline (**4**)

A solution of **3** (200 mg, 0.3 mmole) in 25 ml of MeOH and 2.5 ml of 1N aqueous NaOH was stirred at room temperature for 96 h. The reaction mixture was concentrated *in vacuo*, and the residue was taken up in chloroform (25 ml). The chloroform solution was washed with 3 × 10 ml of water, dried over anhydrous sodium sulfate and concentrated *in vacuo* to give **4** as an oil. The crude product was chromatographed over silica gel with hexane and chloroform (1:3) as eluting solvent; oil; 125 mg (89 %): IR (neat) 1662; ^{1}H-NMR ($CDCl_3$) δ, 1.37 (d, J=6 Hz, 3H, 1′-Ch_3), 1.69 (bm, 4H, H-2′, 3′), 2.73 (t, J=6 Hz, 2H, H-4′), 3.75 (m, 1H, H-1′), 3.96 (s, 3H, OCH_3), 6.26 (s, 1H, H-7), 7.37 (dd, J=4, 8.8 Hz, 1H, H-3), 8.4 (dd, J=1.5, 8.8 Hz, 1H, H-4), 8.5 (dd, J=1,5, 4 Hz, H-2); ^{13}C-NMR ($CDCl_3$) δ, 145.2 (d, C-2), 124.4 (d, C-3), 132.2 (d, C-4), 128.8 (s, C-4a), 103.0 (s, C-5), 163.8 (s, C-6), 89.3 (d, C-7), 150.7 (s, C-8), 134.2 (s, C-8a), 48.5 (d, C-1′), 34.2 (t, C-2′), 30.2 (t, C-3′), 42.2 (t, C-4′), 20.3 (q, C-5′), 56.1 (q, $ArOCH_3$), 186.9 (t, $ArCOCF_2CF_2CF_3$); MS *m/z* 455 (M^+ 6 %), 438 (M^+-H_3N, 5 %), 397 (M^+-$CH_2CH_2CH_2NH_2$, 14 %), 286 (M^+-$CF_2CF_2CF_3$, 65 %), 269 (8 %), 259 (32 %), 201 (100 %). Anal. Calc. $C_{19}H_{20}F_7N_3O_2$: C, 50.11; H, 4.43; N, 9.23. Found: C, 49.87; H, 4.61; N, 8.98.

8-(4-Heptafluorobutyrylamido-1-methylbutylamino)-6-methoxyquinoline (**5**)

A solution of *N,N′*-dicyclohexylcarbodiimide (DCC) (1.13 g, 5.48 mmole) in 10 ml of methylene chloride was added to an ice cold mixture of primaquine (**1**) free base (1.29 g, 5 mmole) and heptafluorobutyric acid (1.17 g, 5.4 mmole) in 30 ml methylene chloride. After complete addition of the DCC solution, triethylamine (0.8 g, 7.9 mmole) was added to the above reaction mixture which was allowed to come to room temperature and further stirred for 48 h. The excess of DCC was decomposed by adding a few drops of acetic acid. The separated solid was filtered off, the solid was washed with a small amount of cold methylene chloride, and the filtrate was concentrated *in vacuo* to give an oil. This oil was chromatographed over silica gel with hexane and chloroform (1:1) as eluting solvent. The earlier fractions gave an oil which was crystallized from a mixture of ether hexane to give colorless solid **5**; mp. 92–93°C; 1.4 g (64 %) [lit. (4) m.p. 88–90°C]: IRλ_{max} (KBr) 1700; ^{1}H-NMR ($CDCl_3$) δ, 1.28 (d, J=6 Hz, 3H, 1′-CH_3), 1.46–1.80 (bm, 4H, H-2′, 3′), 3.33 (bt, 2H, H-4′), 3.50–3.73 (bm, 1H, H-1′), 3.87 (s, 3H, OCH_3), 6.27 (d, J=3, 1H, H-7), 6.33 (d, J=3, 1H, H-5), 7.30 (dd, J=4, 8.8, 1H, H-3), 7.91 (dd, J=1.5, 8.8, 1H, H-4), 8.54 (dd, J=1.5, 4, 1H, H-4); ^{13}C-NMR ($CDCl_3$) δ, 144.6 (d, C-2), 122.0 (d, C-3), 135.0 (d, C-4), 130,2 (s, C-4a), 92.3 (d, C-5), 159.8 (s, C-6), 97.3 (d, C-7), 145.1 (s, C-8), 135.7 (s, C-8a), 48.0 (d, C-1′), 33.9 (t, C-2′), 25.7 (t, C-3′), 40.3 (t, C-4′), 20.6 (q, C-5′), 55.2 (q, $ArOCH_3$), 157.9 (t, $NH\text{-}COCF_2CF_2CF_3$); MS *m/z*, 455 (M^+, 18 %), 440 (M^+-CH_3, 2 %), 286 (M^+-$CF_2CF_2CF_3$, 1 %), 266 (20 %), 201 (100 %). The later fractions gave primaquine *N*-acetate, 300 mg.

8-(4-Amino-1-methylbutylamino)-5-trifluoroacetyl-6-methoxyquinoline (**6**)

A solution of **2** (1.0 g, 2.21 mmole), in 80 ml of 80 % MeOH and 10 ml of 1N aqueous NaOH was stirred at room temperature for 2 hrs. Workup of the reaction mixture as described above for **4** gave **6** as a colorless oil, 0.6 g (76 %). This oil was dissolved in minimum amount of EtOH and treated with 10 % solution of phosphoric acid in ethanol to give **6** as a phosphate salt which was recrystallized from aqueous ethanol, mp. 188–192°, IR λ_{max} phosphate salt (KBr) 1665; ^{1}H-NMR free base ($CDCl_3$) δ, 1.37 (d, J=6 Hz, 3H, 1′ CH_3), 1.53–1.9 (bm, 4H, H-2′-3′), 2.75 (t, J=6 Hz, 2H, H-4′), 3.67–3.93 (m, 1H, H-1′), 4.0 (s, 3H, OCH_3), 6.23 (s, 1H, H-7), 7.40 (dd, J=4, 8.8 Hz, 1H, H-3), 8.51 (dd, J=1.5, 4 Hz, 1H, H-2), 8.69 (dd, J=1.5, 4 Hz, 1H, H-2), 8.69 (dd, J=1.5, 8.8 Hz, H-4); ^{13}C-NMR ($CDCl_3$) δ, 145.1 (d, C-2), 124.5 (d, C-3), 132.7 (d, C-4), 129.1 (s, C-4a), 102.1 (s, C-5), 164.4 (s, C-6), 89.0 (d, C-7), 150.9 (s, C-8), 134.1 (s, C-8a), 48.3 (d, C-1′), 34.1 (t, C-2′), 30.1 (t, C-3′), 42.1 (t, C-4′), 20.3 (q, C-5′), 56.0 (q, $ArOCH_3$), 182.3 (q, Ar $COCF_3$), 117.0 (q, Ar-$COCF_3$); MS, *m/z*, 355 (M_+, 10 %), 338 (M^+-H_3N 22 %), 323 (9 %), 307 (3 %), 297 (m^+-$CH_2CH_2CH_2NH_2$, 100 %), 286 (M^+-CF_3, 15 %), 271 (6 %), 213 (14 %), 201 (39 %). Anal. Calc.: $C_{17}H_{20}F_3N_3O_2 \cdot 2H_3PO$; 37.03; H, 4.75; N, 7.62. Found: C, 37.31, H, 4.56; N, 7.61.

8-(4-Trifluoroacetamido-1-methylbutylamino)-6-methoxyquinoline (**7**)

A solution of *N,N'*dicyclohexylcarbodiimide (DCC) (2.26 g, 10.96 mmole) in 20 ml of methylene chloride was added to an ice cold mixture of primaquine (**1**) free base (2.59 g, 10 mmole) and trifluoroacetic acid (1.24 g, 10.8 mmole) in 60 ml of methylene chloride. After complete addition of the DDC solution, triethylamine (1.6 g, 15.6 mmole) was added to the above reaction mixture which was allowed to come to room temperature. After stirring for 15 h. another lot of trifluoroacetic acid (0.62 g, 5.4 mmole) and DCC (1.13 g, 5.43 mmole) was added to the reaction mixture. The reaction mixture was further stirred at room temperature for another 24 h. and then worked up as described above for **5**. The crude oil was chromatographed over silicia gel with hexane and chloroform (10:90) as eluting solvent. The earlier fractions gave **7** as a colorless solid which was recrystallized from hexane containing a few drops of ether; mp. 87–89°C; 1.8 g (51 %): IR λ_{max} (KBr) 1700; ^{1}H-NMR ($CDCl_3$) δ 1.28 (d, J=6 Hz, 3H, 1'-CH_3), 1.52–1.88 (bm, 4H, H-2', 3'), 3.30 (bt, 2H, H-4'), 3.48-3.72 (m, 1H, H-1'), 3.85 (s, 3H, OCH_3), 6.25, (d, J=3, 1H-7), 6.33 (d, J=3, 1H, H-5), 7.26 (dd, J=4, 8.8 Hz, 1H, H-3), 7.88 (dd, J=1.5, 8.8, 1H, H-4), 8.47 (dd, J=1.5, 4, 1H, H-2); ^{13}C-NMR ($CDCl_3$) δ, 144.2 (d, C-2), 121.9 (d, C-3), 135.1 (d, C-4), 130.1 (s, C-4a), 92.2 (d, C-5), 159.6 (s, C-6), 97.3 (d, C-7), 144.8 (s, C-8), 135.1 (s, C-8a), 47.9 (d, C-1'), 33.8 (t, C-2'), 25,6 (t, C-3'), 40.0 (t, C-4'), 20.5 (q, C-5'), 55.2 (q, $ArOCH_3$), 157.9 (q, NH-$COCF_3$), 116.1 (q, NH-$COCF_3$); MS *m/e*, 355 (M^+ 23 %), 340 (M^+-H_3N, 3 %), 270 (1 %), 241 (2 %), 215 (10 %), 201 (100 %). Anal. Calc. $C_{17}H_{20}F_3N_3O_2$: C, 57.46; H, 5.67; N, 11.82. Found: C, 57.75; H, 5.51; N, 11.49.

Results

Synthesis

Primaquine, on treatment with HFBA under the reaction conditions described by Rai Gopalan et al. (4) gave a product having the same melting point as that reported. However, the spectral characteristics were inconsistent with the previously assigned structure. ^{1}H-NMR of the substance showed a broad triplet at δ 3.30–3.53 for H-4', a sharp singlet at δ 6.23 integrating for one aromatic proton and a signal at δ 8.36 for H-4. The ^{13}C-NMR spectrum (PND) of this product showed a triplet at 158.2 ppm (J_{CCF}=26.3 Hz) for the carbonyl carbon of the heptafluorobutyrylamido group, a second triplet at 187.4 ppm (J_{CCF}=27.3 Hz) for an aromatic carbonyl carbon of a heptafluorobutyryl group, a sharp singlet (PND and SFORD) at 103.5 ppm for C-5 and a doublet at 89.4 ppm for C-7. The carbonyl absorption bands in the IR (λ_{max}) at 1645 and 1691 cm^{-1} were also consistent for the presence of an amide and aromatic carbonyl group. Based on ^{1}H-NMR (downfield shift of H-4) and other spectral evidences this product was assigned structure **3**. Further evidence for this structure was obtained by the synthesis of **4, 5, 6** and **7** and comparison of their spectral data.

Compound **3** on alkaline hydrolysis with 1N sodium hydroxide in 80 % methanol gave **4**. ^{1}H-NMR of **4** showed a triplet at δ 2.73 (J= 6 Hz) for H-4', a sharp singlet at δ 6.26 for H-7 and a signal at δ 8.4 for H-4. The upfield shift of H-4' indicated the hydrolysis of the heptafluoroamido group while the downfield position of H-4 supported the presence of the other heptafluorobutyryl group at C-5. The ^{13}C-NMR also confirmed the structure of **4** as there was one triplet at 186.9 ppm (J_{CCF}=26.4 Hz) for the carbonyl carbon of the heptafluorobutyryl group, a sharp singlet at 103.0 ppm for C-5 and a doublet at 89.3 ppm for C-7. Compound **5** was obtained by dicyclohexylcarbodiimide (DCC) condensation of **1** with heptafluorobutyric acid in methylene chloride in the presence of triethylamine. This compound had the same melting point as reported for the monoheptafluorobutyramide by Raj Gopalan et al. (4). A broad triplet at δ 3.33 was present for H-4' in the ^{1}H-NMR, a doublet at δ 6.33 (J=3 Hz) integrating for one proton for H-5, a doublet at δ 6.27 (J=3 Hz) integrating for one proton for H-7 and a signal at δ 7.91 for H-4. In the ^{13}C-NMR spectrum there was a doublet at 92.3 ppm for C-5, a second doublet at 97.3 ppm for C-7 indicating that there is no substitution of the aromatic ring. The IR spectrum showed the presence of one carbonyl band at (λ_{max}) 1700 cm^{-1}. In this reaction a small quantity of primaquine *N*-acetate was also isolated in addition to **5**. The structure of the *N*-acetate was confirmed by the comparison with an authentic sample. The formation of *N*-acetate of primaquine can be explained on the basis of condensation of **1** with acetic acid during work up of the reaction.

The synthesis of **6** was carried out by the alkaline hydrolysis with 1N NaOH of **2** which was obtained as described earlier (3). In ^{1}H-NMR **6** showed a triplet at δ 2.75 (J=6) for H-4', a sharp singlet at δ 6.23 for H-7 and a signal at δ 8.69 for H-4. The ^{13}C-NMR was also consistent with structure **6** as there was a singlet at 102.1 ppm for C-5, a doublet at 89.0 ppm for C-7, a quartet at 117.0 (J_{CF}= 291 Hz) for the carbon of the trifluoromethyl group and a quartet at 182.3 ppm (J=35.1 Hz) for carbonyl carbon of the aromatic trifluoroacetyl group. The amide **7** was obtained by DCC condensation of **1** with trifluoroacetic acid in the presence of triethylamine as described for **4**. In ^{1}H-NMR **7** showed a signal at δ 3.30 for H-4', a signal at δ 6.29 integrating two protons for H-5,7 and a doublet at 7.88 (J=1.5, 8.8 Hz) for H-4. The ^{13}C-NMR of **7** also confirmed the assigned structure.

Biological Evaluation

To determine the effect of acylation of the 5 position of primaquine on biological activity as an antimaterial, **6** as its diphosphate salt, was submitted to Dr. Wallace Peters, Department of Medical Protozoology, London School of Hygiene and Tropical Medicine, London England for evaluation of its biological activity as a causal prophylactic against sporozoite induced infections of mice by *Plasmodium yoelii nigeriensis* and for its blood schizontocidal activity in mice against *Plasmodium berghei*. Dr. Peters directs a program of evaluation of substances under the sponsorship of the World Health Organization. The substance showed marginal activity as a blood schizontocide (approximately one third to one tenth that of primaquine) and essentially no activity as a causal prophylactic agent. These results would suggest that substitution of primaquine with an electronegative grouping in the 5 position is detrimental to activity as an antimalarial agent.

Acknowledgements
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References

(1) McChesney J. D., Sarangan S. (1984) Pharm. Res. 184–186.

(2) Clark A. M., Evans S. L., Hufford C. D., McChesney J. D. (1982) J. Nat. Prod. 45, 574–581.
(3) Clark A. M., Hufford C. D., Gupta R. C., Puri R. K., MyChesney J. D. (1984) Appl. Environ. Micro. 47, 537–539.
(4) Raj Gopalan T. G., Anjaneyulu B., Shanbag V. D., Grewal R. S. (1981) J. Chromatogr. 224, 265–273.
(5) Clark A. M., Hufford C. D., McChesney J. D. (1981) Antimicrob. Ag. Chemother. 19, 337–341.

Anticonvulsant and Monoamine Oxidase Inhibitory Activities of some Triazene N^1-Oxides

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Abstract: Thirty-three N^3-2-, -3- or -4-substituted aryl-N^1-(alkyl/aryl/substituted aryl)-triazene N^1-oxides were synthesized and evaluated for their anticonvulsant and monoamine oxidase (MAO) inhibitory activities. Most of the compounds exhibited MAO inhibitory activity *in vitro*, and kinetic studies conducted with N^3-4-chlorophenyl-N^1-methyltriazene N^1-oxide, the most potent inhibitor, showed that the inhibition is non-competitive in nature. The MAO inhibiting activity of the compounds correlated well with their anticonvulsant effect against maximal electroshock-induced seizures in rats. Acute toxicity studies indicate that the compounds have a wide margin of safety.

Monoamine oxidase inhibitors are known to exhibit significant anticonvulsant activity against experimentally induced seizures, and a significant correlation, between these two activities has been established (1–4), although reports to the contrary also exist (5, 6). Triazene N^1-oxides have been recently reported to exhibit significant anticonvulsant activity against maximal electroshock-induced seizures in rats (7). In this communication we report the *in vitro* MAO inhibitor activity of a series of triazene N^1-oxides, synthesized in this laboratory, together with their anticonvulsant effects, as a function of their chemical structures.

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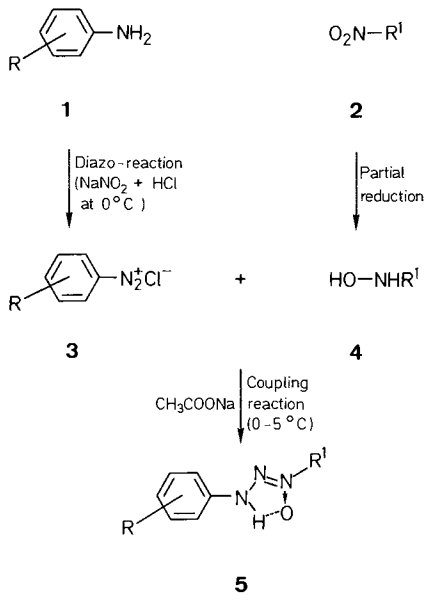
The compounds were synthesized as per the route shown in Fig. 1. The diazonium salt (**3**), prepared from the corresponding primary aryl amine (**1**), when coupled with hydroxylamine (**4**), prepared from the corresponding nitro-compound (**2**), in acetate buffered solution, yielded the corresponding triazene N^1-oxide (**5**). The structures of the compounds were established by elemental analysis, IR and NMR spectral data. The analytical results for elements were within ± 0.4 % of the theoretical values. The prepared compounds are listed in Table I, along with their physical constants.



Materials and Methods

Synthesis of N^3-phenyl-N^1-methyl-triazene N^1-oxide:

Freshly distilled aniline (0.06 mole) was diazotized, the reaction taking approximately 30 min for completion, and nitromethane (0.15 mole) was reduced with zinc dust (22.7 g) and ammonium chloride (9.1 g) to methylhydroxylamine (0.10 mole), in an ice bath with constant stirring. The diazotized product was slowly added to this latter solution, with occasional addition of a saturated solution of sodium acetate (40 g), to maintain the pH at 4.5 to 5.5, and crushed ice to keep the temperature between 0 to 5° C. A creamy yellow mass separated, which was filtered under suction, washed with ice cold water and crystallized from aqueous alcohol. It was finally decolorized with charcoal to yield silky white crystals. M.p.: 71° C; yield: 4 g (44 %); IR (KBr cm^{-1}) : 3160 (–NH), 1520 (N→0), 1440 (–N=N–), 1320 (–N=N–NH–); NMR ($CDCl_3$ δ scale): 3.9 (s, 3H, N–CH_3), 6.8 to 7.4 (m, 5H, Ar-H), 10.1 (bs, 1H, –NH).

The other substituted triazene N^1-oxides were prepared similarly, using appropriate molar quantities of the corresponding amino and nitro compounds. Benzocaine derivatives were unstable at pH 4.5 to 5.5 and were prepared at a lower pH (1.0 to 2.5).

Enzymatic and Pharmacological Assays

MAO inhibitory activity was determined by a radiometric enzyme assay technique (8). The enzyme source was rat liver homogenate and the substrate used was ^{14}C-tyramine. The test drugs were dissolved in propylene glycol, and the MAO inhibition afforded by graded concentrations of the compounds was determined with the respect to controls, where only equivalent amount of propylene glycol was added to the reaction mixture. The I_{50} values of the test compounds were calcualted as the concentration required to induce 50 % inhibition of the enzyme activity. In a separate study, the nature of enzyme inhibition,