# N-Oxides and S-Oxides of Ellipticine Analogs (1)

Allan N. Fujiwara, Edward M. Acton, and Leon Goodman

Life Sciences Research, Stanford Research Institute

Oxidation with perbenzoic acid derivatives in neutral organic solvents of 11-desmethylellipticine (at the pyrido-N) and oxa-ellipticine afforded the corresponding N-oxides. Thia-ellipticine afforded the sulfone N-oxide, which underwent N-deoxygenation with triphenylphosphine to yield thia-ellipticine sulfone.

The peracid oxidation of several ellipticine analogs was undertaken in order to study the effect on antitumor activity in this series of having the pyrido-nitrogen present as an N-oxide. Previous examples of aromatic N-oxides which have shown anticancer activity are a number of purine N-oxides (2) and some N-oxides of 4-nitroquinolines (3). Dose levels were important with these compounds, as many were carcinogenic at high levels. In some cases, the carcinostatic properties were attributed to activity of the parent compound, regenerated by deoxygenation *in vivo*. In the present work, N-oxides (II and V) have been prepared from 11-desmethylellipticine (I) (4) and oxa-ellipticine (III) (5); thia-ellipticine (IV) (6) underwent oxidation at both nitrogen and sulfur to give the sulfone N-oxide (VI), which by chemical deoxygenation afforded the sulfone (VII). Comparison of the biological activities with those of the parent heterocycles should be instructive as to the effect of the oxide function. 11-Desmethyl-ellipticine and thia-ellipticine as parent compounds have shown good antitumor activity (4, 5), whereas oxa-ellipticine was inactive.

The optimum oxidizing conditions were the use of *meta*-chloro- and *para*-nitroperbenzoic acids, in excess, in neutral solvents. In 1,2-dichloroethane, the conversion of oxa-ellipticine (III) to V was essentially complete. Hexamethylphosphorotriamide was the solvent of choice for desmethylellipticine (I) because of its insolubility in other solvents, but even then the oxidation could not be carried to completion; the N-oxide (II) had to be separated, chromatographically, or by virtue of its relative insolubility in aqueous acid, from unreacted I. The N-oxides (II and V) showed characteristic, strong infrared bands at 8.2-8.3 μ (7). The trioxide (VI), prepared from thia-ellipticine in dichloroethane, showed strong sulfone bands at 7.8 and


III, X = O
IV, X = S

V

VI

VII
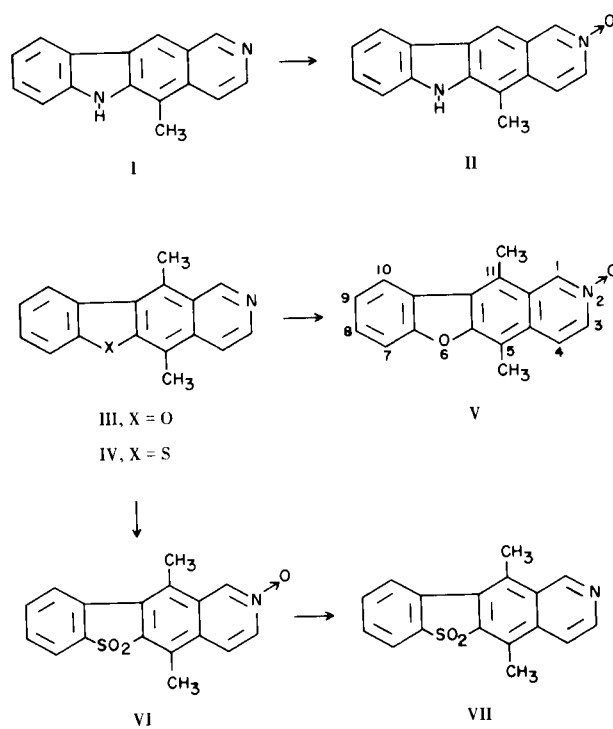
8.6 μ in addition to N-oxide absorption at 8.13 μ. Loss of the N-oxide and retention of the sulfone bands in the infrared was diagnostic for N-deoxygenation to the sulfone (VII), upon fusion of the trioxide (VI) with triphenylphosphine at 260°. These oxides all were crystalline and chromatographically distinct from their precursors.

With oxa-ellipticine (III), the N-oxidation could also be characterized by nmr studies, since the N-oxide (V) was soluble in deuteriochloroform and could be compared with III (data in ref. 5). The proton at lowest field for both III and V was H-1. It was characteristic of an N-oxide that for

V the H-1 signal (δ 9.10) was shielded relative to H-1 for III (δ 9.57), at least in aprotic solvents. Less obvious but equally significant was that H-3 also was shielded in going from III (δ 8.48) to V (δ 8.17), whereas H-4 was somewhat deshielded (from δ 7.62 to 7.78, respectively). Protons α to a heterocyclic nitrogen (e.g., H-1 or H-3) undergo a higher shift and β-protons (e.g., H-4) a lower shift, on N-oxidation (8). An increase of about 1 Hz in the coupling constant, $J_{3,4}$, for the N-oxide (V, 7.5 Hz) relative to oxa-ellipticine (III, 6.0 Hz) was characteristic for the development of a positive charge on nitrogen in an isoquinoline system, as in protonation, quaternization, or N-oxidation (9). Proton resonance of the desmethyl N-oxide (II) was not studied because of its low solubility.

As preliminary model experiments, 1,4-dimethyldibenzothiophene (6) and the tetrahydro derivative (6) were oxidized to the corresponding sulfones (VIII and IX) with hydrogen peroxide in glacial acetic acid (10). These were not useful oxidizing conditions for desmethylellipticine (I) or oxa-ellipticine (III), however, as there was little or no attack at nitrogen. Somewhat surprisingly, then, thia-ellipticine (IV) was readily oxidized in hydrogen peroxide-acetic acid to the sulfone N-oxide (VII) accompanied by over-oxidation of the pyrido ring to give a nitrogen-free by-product. This was identified as the anhydride (X) by the elemental analysis, and by characteristic carbonyl bands at 5.42, 5.50 (medium) and 5.63 μ (strong) in the infrared; sulfone bands were present at 7.67 and 8.59 μ, but there was no absorption at 8.1-8.2 μ for an N-oxide. The simple analog, 3,6-dimethylphthalic anhydride showed carbonyl absorption at 5.44 and 5.70 μ (11). Presence of the two methyls in X was verified by the presence of two singlets in the nmr spectrum. The isolation of X, through conversion with methanolic sodium methoxide to a water soluble residue, presumably a sodium salt, also supported the anhydride structure; acidification of the basic solution (after removal of VI on a filter) regenerated X. 3,6-Dimethylphthalic anhydride similarly precipitated (11) from
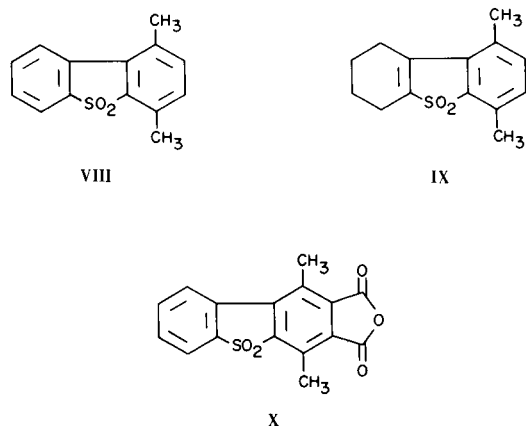
aqueous solutions of the corresponding diacid or monoacid ester. No trace of X was detected in the oxidation of thia-ellipticine in dichloroethane with p-nitroperbenzoic acid.

The sulfones (VIII and IX), unlike the parent dibenzothiophenes (6), underwent no reaction at all with stannic chloride-butyl dichloromethyl ether, in an attempt to form aldehydes which might have been utilized for the attachment of a pyrido ring in other syntheses. The deactivating effect of the sulfone function in dibenzothiophenes has been noted (12).

EXPERIMENTAL

Methods.

Melting points were determined on a Fisher-Johns hot stage and are uncorrected. Uv spectra were determined with a Cary Model 11 recording spectrophotometer. Infrared spectra were determined in Nujol mull; only bands useful for structure assignments are reported. Nmr spectra were determined with Varian A-60 and HA-100 spectrometers; tetramethylsilane (δ 0.00) was the internal reference standard in chloroform-d, external in trifluoroacetic acid and pyridine-d_5. Signals are described as singlet (s), doublet (d), or multiplet (m). Accuracy is ± 0.05 ppm for chemical shifts.

Integrated signal ratios were as expected from the structure assignments. Thin-layer chromatography (tlc) was done on silica gel G and the spots detected under uv light.

5-Methyl-6H-pyrido[4,3-b]carbazole-2-oxide (II, 11-Desmethyl-ellipticine N-oxide).

A solution of 115 mg. (0.50 mmole) of 11-desmethyl-ellipticine (i.e., 1-desmethylolivacine, I) (4) in 3 ml. of hexa-methylphosphortriamide was treated with 173 mg. (1.00 mmole) of m-chloroperbenzoic acid, heated for 16 hours at 75°, and poured into 30 ml. of water, whereupon the product precipitated. The suspension was basified (pH 10) with sodium hydroxide solution and stirred to dissolve any acid, and the solid was collected on a filter and washed with water. It weighed 98 mg. and was a mixture of unreacted I (R_f 0.8 on tlc in methanol-chloroform, 1:9) and N-oxide (II) (R_f 0.4), according to tlc and infrared spectral data. Preparative tlc on two 20 x 20-cm. plates (2-mm. layer of silica gel) afforded 33 mg. (29%) of recovered I and 41 mg. (33%) of N-oxide, m.p. 220-265° dec. Alternatively, unreacted I was removed from the crude product by trituration with warm 1 M acetic acid, whereupon I dissolved (along with a small amount of II) and most of the N-oxide II remained insoluble (49% yield, homogeneous on tlc), m.p. 205-228° dec. The oxidation could not be driven to completion, even after three days, with more peracid added daily. Recrystallization from 95% ethanol (50 ml./g.) afforded the analytical sample, m.p. 238-241° dec.; uv (ethanol) (ε x 10⁻³) 234 (19.9), 253 (20.3), 293 (41.8), 303 (42.3), 314 (65.6), 347 mμ (5.95); ir μ 6.21 (strong, aryl), 8.10 (also present in I, strong, unassigned), 8.28 (strong, N-oxide), 8.60-8.68 (strong, unassigned; outside the usual N-oxide range).

Anal. Calcd. for $C_{16}H_{12}N_2O \cdot 1/3H_2O$: C, 75.6; H, 4.87; N, 11.0. Found: C, 75.5; H, 4.98; N, 10.9.

5,11-Dimethylbenzofuro[2,3-g]isoquinoline-2-oxide (V, Oxa-ellipticine N-oxide).

VIII   IX

X

A mixture of 3.7 g. (15 mmoles) of oxa-ellipticine (III) (5) and 5.5 g. (30 mmoles) of p-nitroperbenzoic acid in 375 ml. of 1,2-dichloroethane was refluxed for 24 hours, when tlc of an aliquot in heptane-ethylacetate-methanol-water (3:10:6:5, upper phase) showed that the N-oxide (R_f 0.2) contained only a trace of unoxidized III (R_f 0.7), which however remained after further refluxing with 2.7 g. of fresh peracid. The product was best freed of the acids, after removal of solvent in vacuo, by trituration with 125 ml. of 5% sodium hydroxide. The product was washed with water (if gummy, it was first dissolved in dichloromethane) until free of base. When dried, it weighed 3.5 g. (89%), m.p. 235-263° dec. Recrystallization from 30 ml. of methanol, with addition of a few ml. of hexane and ether, afforded 1.8 g. (45%) in two crops, m.p. 253-265° dec.; ir (Nujol) μ 6.17, 6.22, 6.32 (weak, aryl; oxa-ellipticine showed a single aryl band at 6.22 μ of medium intensity), 8.27 (strong, broad, N-oxide; oxa-ellipticine showed a sharp strong band at 8.21 μ); nmr (deuteriochloroform) δ 9.10 d (H-1, $J_{1,3}$ = 1.8 Hz), 8.17 q (H-3, $J_{1,3}$ = 1.8 Hz, $J_{3,4}$ = 7.5 Hz), 8.17-7.94 m (H-10), 7.78 d (H-4, $J_{3,4}$ = 7.5 Hz), 7.70-7.27 (H-7, H-8, H-9), 2.90 s (1-CH$_3$), 2.73 s (4-CH$_3$). The analytical sample was recrystallized from 1,2-dichloroethane-methanol (5:1, 50 ml./ g.; 60% recovery) and dried at 140° in vacuo for 2 days, m.p. 260-268° dec.

Anal. Calcd. for $C_{17}H_{13}NO_2$: C, 77.6; H, 4.98; N, 5.32. Found: C, 77.6; H, 5.01; N, 5.29.

5,11-Dimethylbenzothieno[2,3-g]isoquinoline-2,6,6-trioxide (VI, thia-ellipticine sulfone N-oxide).

To a stirred solution of 1.82 g. (6.92 mmoles) of thia-ellipticine (IV) (6) in 175 ml. of 1,2-dichloroethane was added 5.00 g. (27.3 mmoles) of p-nitroperbenzoic acid, and the suspension was refluxed for 16 hours. After chilling, the solid was collected. It was suspended in 60 ml. of 5% sodium hydroxide and stirred to remove acids, then was collected and washed with water until the washings were neutral. Dried in vacuo at 140° overnight, it weighed 1.51 g. (70%), m.p. 295-299° dec.; ir (Nujol) μ 6.18 and 6.28 (weak, aryl), 7.75 (strong, SO$_2$), 8.13 (strong, N-oxide), 8.33 (medium, unassigned; there was no absorption at 8.23 μ where thia-ellipticine showed an unassigned band of medium intensity), 8.61 (strong, SO$_2$), 8.87 (medium, SO$_2$); nmr (trifluoroacetic acid) δ 9.42 s (H-1), 8.19 s (H-3 and H-4), 8.05-7.80 m (H-10), 7.75-7.25 m (h-7, H-8, H-9), 2.72 s and 2.68 s (H-5 and H-11). It was homogeneous on tlc, R_f 0.6 in methanol, R_f 0.3 in heptane-ethyl acetate-methanol-water (3:10:6:5, upper phase) where it was best distinguished from IV of R_f 0.7.

Anal. Calcd. for $C_{17}H_{13}NO_3S\cdot1/2H_2O$: C, 63.7; H, 4.40; N, 4.37. Found: C, 63.8; H, 4.14; N, 4.36.

A sample recrystallized from glacial acetic acid (10 ml./g.) afforded a solvated sample (50% recovery), according to ir and nmr spectral evidence for C=O and acetyl CH$_3$, respectively.

5,11-Dimethylbenzothieno[2,3-g]isoquinoline-6,6-dioxide (VII, thia-ellipticine sulfone).

An intimate mixture of 886 mg. (2.85 mmoles) of sulfone N-oxide (VI) and 815 mg. (3.10 mmoles) of triphenylphosphine was fused and heated under nitrogen at 255-260° (oil bath) for 45 minutes. The cooled solid was ground in a mortar and triturated with two 25-ml. portions of hot hexane (to remove triphenylphosphine) and with two 12-ml. portions of warm ethanol (to remove triphenylphosphine oxide). The insoluble product, 716 mg. (85%), m.p. 230-261° dec., was homogeneous on tlc (R_f 0.6 in methanol-benzene, 1:9, vs. R_f 0.3 for sulfone oxide VI) except for trace contamination near the origin. The infrared spectrum was identical to that of an analytical sample obtained (60% yield

in two crops) by recrystallization from chloroform-carbon tetrachloride (1:1); complete absence of absorption at 7.9-8.4 μ was diagnostic for absence of sulfone N-oxide (VI) and of thia-ellipticine (IV). Drying at 200° in vacuo for 2 days was required for removal of solvent from VII, m.p. 225-255° dec., uv (ethanol) (ϵ x 10^{-3}) 260 (45.5), 291 (11.1), 303 (15.0), 315 mμ (15.1); ir (Nujol) μ 6.27 (medium, aryl), 7.73 and 8.59 (strong, SO$_2$), 8.81 (medium, SO$_2$); nmr (deuteriochloroform) δ 9.62 s (H-1), 8.67 d (H-3, J = 5.8 Hz), 8.23-7.50 m (H-10, H-4, H-7, H-8, H-9), 7.01 s and 6.98 s (5-CH$_3$ and 11-CH$_3$).

Anal. Calcd. for $C_{17}H_{13}NO_2S$: C, 69.1; H, 4.44; N, 4.74. Found: C, 68.9; H, 4.31; N, 4.84.

1,4-Dimethyldibenzothiophene-5,5-dioxide (VIII).

The procedure for the 2,8-dimethyl isomer (10) afforded VIII, which crystallized from the reaction medium and was recrystallized from ethanol, in 82% yield, m.p. 195-198°; ir μ 7.78, 8.57-8.67, 8.82 (SO$_2$).

Anal. Calcd. for $C_{14}H_{12}O_2S$: C, 68.8; H, 4.95; S, 13.1. Found: C, 68.8; H, 4.92; S, 13.1.

1,2,3,4-Tetrahydro-6,9-dimethyldibenzothiophene-5,5-dioxide (IX).

Similarly, IX was obtained in 53% yield, m.p. 196-199°; ir μ 7.78, 8.63, 8.82 (SO$_2$).

Anal. Calcd. for $C_{14}H_{16}O_2S$: C, 67.7; H, 6.49; S, 12.9. Found: C, 67.5; H, 6.58; S, 12.9.

1,4-Dimethyl-2,3-dibenzothiophenedicarboxylic Anhydride 5,5-Dioxide (X).

A sample (0.89 g.) of crude sulfone N-oxide (VI), obtained from IV with 30% hydrogen peroxide in glacial acetic acid and contaminated with X (evidenced by a sharp ir band of medium intensity at 5.62 μ), was suspended in 75 ml. of methanol containing 0.24 g. of sodium methoxide. After refluxing for 24 hours, the methanol was removed and the residue was triturated with water. Insoluble VI (0.44 g.) was removed on a filter, and the basic filtrate was acidified to pH 2. The precipitate was collected, 0.39 g., m.p. 175-270°. Recrystallization of 140 mg. from 7 ml. of tetrahydrofuran and 0.5 ml. of water afforded 57 mg., m.p. 296-302°; ir μ 5.42, 5.50, 5.62 (C=O, anhydride), 7.67 and 8.59 (SO$_2$), 7.98 and 8.33 (unassigned); nmr (pyridine-d$_5$) δ 2.41 s and 2.07 s (two aryl CH$_3$'s; aryl H's near δ 7.0 were obscured by solvent impurities). This analytical sample was chromatographically identical (R_f 0.5 in methanol) to the impurity in crude VI (R_f 0.9 for VI).

Anal. Calcd. for $C_{16}H_{10}O_5S$: C, 61.1; H, 3.21; S, 10.2. Found: C, 61.2; H, 3.02; S, 10.5.

REFERENCES

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. PH-43-64-500. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center.

(2) K. Sugiura, *Cancer Chemotherapy Rpts.*, 1, 383 (1968), and leading references.

(3) E. Ochiai, "Aromatic Amine Oxides," Elsevier Publishing Co., Amsterdam, London, New York, 1967, pp. 435, 437.

(4) C. W. Mosher, O. P. Crews, E. M. Acton, and L. Goodman, *J. Med. Chem.*, 9, 237 (1966), wherein this compound is

alternatively named 1-desmethylolivacine.

(5) A. N. Fujiwara, E. M. Acton, and L. Goodman, *J. Heterocyclic Chem.*, **6**, 379 (1969).

(6) A. N. Fujiwara, E. M. Acton, and L. Goodman, *ibid.*, **5**, 853 (1968).

(7) A range of 1300-1200 cm^{-1} (*i.e.*, 7.69-8.33 μ) has been suggested; ref. 3, p. 114.

(8) Reference 3, p. 109.

(9) M. H. Palmer and B. Semple, *Chem. Ind.* (London), 1766 (1965).

(10) R. Gerdil and E. A. C. Lucken, *J. Am. Chem. Soc.*, **87**, 213 (1965).

(11) L. Eberson, *Acta Chim. Scand.*, **18**, 2015 (1964).

(12) N. M. Cullinane, C. G. Davies, and G. I. Davies, *J. Chem. Soc.*, 1435 (1936).