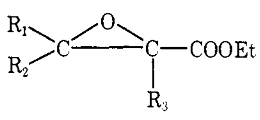
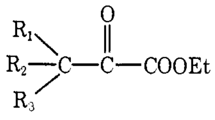
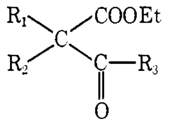
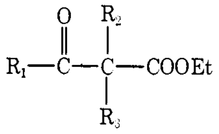
The scope and mechanism of these rearrangements of glycidic esters are being studied under acidic, thermal, and photochemical conditions.


I


II


III


IV

a, $R_1 = C_6H_5$; $R_2 = R_3 = H$
b, $R_1 = C_6H_5$; $R_2 = CH_3$; $R_3 = H$
c, $R_1 = C_6H_5$; $R_2 = H$; $R_3 = CH_3$
d, $R_1 = C_6H_5$; $R_2 = R_3 = CH_3$
e, $R_1 = R_2 = H$; $R_3 = C_6H_5$
f, $R_1 = CH_3$; $R_2 = H$; $R_3 = C_6H_5$
g, $R_1 = R_2 = CH_3$; $R_3 = C_6H_5$

Acknowledgments. We are grateful to the National Science Foundation for support of this work and S. P. S. thanks the Chemistry Department of the University of Kurukshetra (India) for a leave of absence. We also thank Dr. H. O. House for informing us that he has reexamined the spectral properties of his sample of the 2,4-DNP derivative, and that he is in agreement with our formulation of IIIb for the rearrangement product from Ib.

S. P. Singh, Jacques Kagan
Chemistry Department, University of Illinois at Chicago Circle
Chicago, Illinois 60680

Received August 8, 1969

An Unusual Reaction of Skatole with Tetranitromethane

Sir:

Tetranitromethane (TNM) is a reagent which selectively nitrates tyrosine residues in proteins at pH 8.[1] Because of the apparent specificity of the reagent and the mild reaction conditions, many proteins have been modified by this technique.[2]

Recently modification studies on staphylococcal nuclease[3] and papain[4] have shown that a tryptophan residue also reacts with TNM. Likewise the two vinyl groups of ferriheme at pH 8 react slowly with TNM.[5]

We have studied the mode of reaction of TNM with skatole (**1**). When equimolar proportions of reactants were mixed in diethyl ether at room temperature, orange-red needles (mp 137–141° dec, yield ~50%) separated gradually during the reaction (8–12 hr). Additional crystals (*ca.* 10%) as well as several Ehrlich-positive compounds[6] were obtained on concentration of the mother liquor.

The nmr spectrum (60 MHz, DMSO-d_6) of this product indicated no further substitution of the benzene ring and showed a slight upfield shift ($\Delta\delta = 0.15$ ppm) for the three-proton methyl singlet originally present. The ir spectrum ($CHCl_3$) indicated apparent doublets for the nitro group peaks at 1590, 1525 (asymmetric stretching) and 1333, 1313 cm^{-1} (symmetric stretching) and the absence of the indole N-H stretching band.

The ready solubility in 0.1 *N* NaOH or alkaline buffers and the strong uv absorption at λ_{max}^{EtOH} 395 nm[7] suggested the presence of a dinitromethylene chromophore in conjugation with an acidic proton, such as $-(-NH)-C{=}C(NO_2)_2$.[8]

The highest significant peak in the mass spectrum (Hitachi RMU-6E instrument with 250° inlet, 80-eV ionizing potential) was an ion at *m/e* 251, clearly not the molecular ion, since the combustion analysis (*Anal.* Found: C, 43.23; H, 2.99; N, 19.65) gave an incompatibly high nitrogen value. Reduced inlet temperatures, lower ionization potentials, or chemiionization techniques failed to produce the molecular ion. The thermal instability of this dinitromethylene derivative makes analysis by mass spectroscopy unfeasible.

On the basis of combustion analysis and the molecular weight (285), determined by osmometry in tetrahydrofuran, we consider $C_{10}H_8N_4O_6$ (280) (Calcd: C, 42.86; H, 2.88; N, 20.00) the most likely formula. Structure **2** would agree with the above data and the known TNM reactions in which a dinitromethylene unit is incorporated into the substrate.[9–11]

Since electrophilic substitution of 3-alkylindoles is usually initiated by attack at position 3,[12] the formation of **2** can be rationalized by the sequence **1** → **1a** → **1b** → **2** in which the reactive indolenine **1a** adds the trinitromethane anion followed by elimination of nitrous acid.

This mechanism resembles the reaction of cyclic olefins with TNM.[9] Whether or not π-complex formation precedes or accompanies the formation of **2**[13] or whether a radical pathway[14] is involved cannot be answered at this time.

Reduction of **2** in DMSO–1.0 *M* phosphate (pH 8.0), 1:10 (v/v), with excess $Na_2S_2O_4$ led to a nitrile (mp 104–105°; $\lambda\lambda_{max}^{EtOH}$ 287, 300 (sh), 312 (sh) nm (log $\epsilon\epsilon$ 4.21, 3.97, 3.69); *m/e* 156 (M^+), 155 (base peak); $\nu_{max}^{CHCl_3}$ 3460, 2220 cm^{-1}; δ_{CH_3} (singlet) 2.50 ppm) in 75% yield.

This nitrile was identified as the previously unreported 2-cyano-3-methylindole (**3**) by methylation[15] to the known 1-methyl derivative (**4**)[16] (mp 69.5–71.5°; *m/e* 170 (M^+), 169 (base peak)).

This interesting and potentially useful conversion might possibly go through an enediamine which could

---

(1) M. Sokolovsky, J. F. Riordan, and B. L. Vallee, *Biochemistry*, **5**, 3582 (1966).

(2) See L. A. Cohen, *Ann. Rev. Biochem.*, **37**, 698 (1968), for a brief review.

(3) P. Cuatrecasas, S. Fuchs, and C. B. Anfinsen, *J. Biol. Chem.*, **243**, 4787 (1968).

(4) K. Morihara and K. Nagami, *J. Biochem.* (Tokyo), **65**, 321 (1969).

(5) M. Z. Atassi, *Biochim. Biophys. Acta*, **177**, 663 (1969).

(6) The modified Ehrlich spray, 0.5% *p*-dimethylaminocinnamaldehyde in 1.0 *N* HCl, was used. The major product, a bright yellow spot (R_f 0.62) on silica gel G tlc (toluene–ethyl formate–formic acid, 5:3.5:1), was Ehrlich negative.

(7) The addition of base did not significantly change the uv spectrum. On the basis of a molecular weight of 280, a molar extinction coefficient of 17,200 at 395 nm in EtOH can be calculated.

(8) W. E. Thun, D. W. Moore, and W. R. McBride, *J. Org. Chem.*, **31**, 923 (1966).

(9) R. W. Bradshaw, *Tetrahedron Letters*, 5711 (1966).

(10) D. H. Reid, W. H. Stafford, and W. L. Stafford, *J. Chem. Soc.*, 1118 (1958).

(11) Reference 8 and four references cited therein.

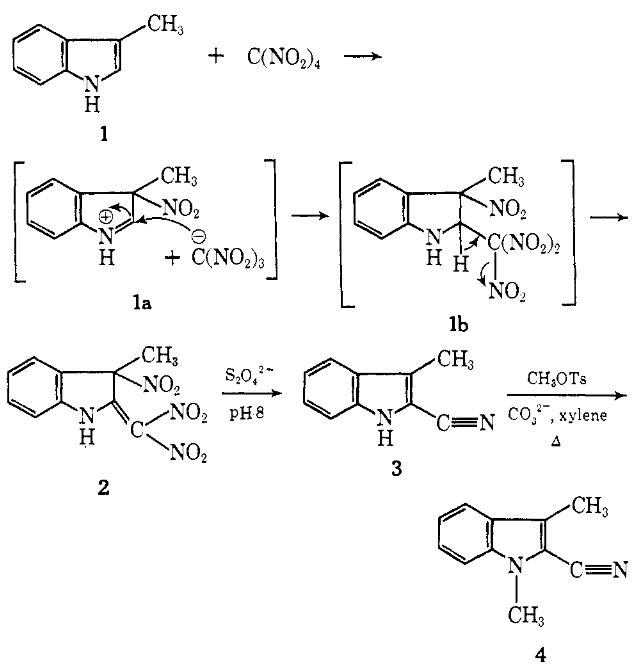
(12) K. M. Biswas and A. H. Jackson, *Tetrahedron*, 227 (1969).

(13) D. H. Iles and A. Ledwith, *Chem. Commun.*, 364 (1969).

(14) T. C. Bruice, M. J. Gregory, and S. L. Walters, *J. Am. Chem. Soc.*, **90**, 1612 (1968).

(15) D. A. Shirley and P. A. Roussel, *ibid.*, **75**, 375 (1953).

(16) H. R. Snyder and E. L. Eliel, *ibid.*, **70**, 1857 (1948).



lose ammonia to give a ketenimine, followed by isomerization and expulsion of nitrite

$$=C(NO_2)_2 \longrightarrow =C(NH_2)_2 \xrightarrow{-NH_3} =C=NH$$

When the reaction with TNM was carried out in aqueous ethanol, significantly decreased yields of **2** resulted, suggesting alternate reaction pathways. In preliminary experiments, TNM on tryptophan peptides in aqueous solvents gave a mixture of nitrated products with the spectral characteristics of nitroindoles.[17] We are now looking into the reaction of tryptophan peptides with TNM in organic solvents.

---

(17) G. Berti, A. Da Settimo, and D. Segnini, *Gazz. Chim. Ital.*, **90**, 539 (1960).

(18) Associate in the Visiting Program of the U. S. Public Health Service, 1968–1969.

Thomas F. Spande, Angelo Fontana,[18] Bernhard Witkop
National Institute of Arthritis and Metabolic Diseases
National Institutes of Health, Bethesda, Maryland 20014

Received July 31, 1969

Preparation and Properties of Crystalline Permanganic Acid[1]

Sir:

Aqueous $KMnO_4$ has been used successfully as an alternative to aqueous OsO_4 for electron microscopy fixation of biological objects.[2] This prompted us to determine if an analog could be prepared that would similarly complement the use of the volatile OsO_4 for vapor-phase fixations.[3] $HMnO_4$ seemed a possibility but had been reported to be too unstable to prepare, and previous attempts at preparation had yielded only products grossly contaminated with MnO_2,[4] or a 2.6 *M* solution which froze at −11° to a mass of ice and $HMnO_4$ crystals.[5]

By analogy with $HClO_4$, it was anticipated that $HMnO_4$ and/or some of its hydrates might be both stable and volatile[6] at low temperature, *e.g.*, 1°. Accordingly, we devised a low-temperature method of preparation depending on fractional, vacuum sublimation of frozen, aqueous $HMnO_4$ which promised to yield both anhydrous and hydrated $HMnO_4$.

Samples of H_2SO_4 and recrystallized $Ba(MnO_4)_2$ were analyzed by the usual gravimetric procedures to ±0.1 %. To 120 ml of 0.3 *M* aqueous $Ba(MnO_4)_2$ at 0° was added a precisely equivalent amount of 0.3 *M* H_2SO_4, care being taken that the temperature remained below 1°, and the resultant precipitate removed by centrifugation. The deep violet, aqueous, $HMnO_4$ was transferred to a 500-ml round-bottom flask, and promptly frozen onto its walls by rotation in a CO_2–acetone bath at −75°. The flask was then connected to a glass and Teflon vacuum system and immersed in an ice bath. The vacuum system, operating through successive −75° CO_2–acetone and −193° liquid N_2 traps, was capable of maintaining a vacuum of 10^{-3} Torr. Ice immediately formed in the −75° trap, indicating removal of water.

After about 10 hr a violet color appeared in the −75° trap, and the system was promptly shunted through a U tube immersed in a −75° bath to collect the violet fraction. This crystallized in the form of fine, deep violet, needles.

After about 30 hr all of the volatile, violet fraction had been removed, and the flask temperature rose to that of the ice bath, indicating an end to vaporization of volatile components. The contents of the flask and the traps were removed for analysis; all operations were conducted at, or below, 1°. In addition, all operations on the flask contents were performed in a drybox, since the material proved quite hygroscopic.

Samples of the rectangular crystals remaining in the flask were extracted with successive portions of cold water. The soluble fraction, on analysis, proved to be pure $HMnO_4$ (see Table I). The insoluble fraction, on analysis, proved to be MnO_2, about 0.1 mole % in all. The U-tube contents were completely water soluble and proved to be $HMnO_4 \cdot 2H_2O$ (Table I). Analyses were performed conventionally except that permanganic acid samples were added quickly to excess reagent, and the

Table I

	$HMnO_4$ 119.944[a]	$HMnO_4 \cdot 2H_2O$ 155.974[a]
Yield, mole %[b]	50.9 ± 0.2	49.0 ± 0.2
Neutralization weight[c]	120.03	156.11
Fe^{2+} titration weight[c]	119.32	155.71
MnO_2 production weight[c]	119.69	156.08
O_2 evolution weight[c]	119.90	156.17
Experimental mean weight	119.74 ± 0.27	156.02 ± 0.18

[a] Theoretical formula weight. [b] Mean of 11 runs. [c] Mean of duplicate determinations.

---

(1) Work supported by U. S. Atomic Energy Commission and presented, in part, at the 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1968, Inorganic Division.

(2) T. N. Tahmisian, R. L. Devine, B. J. Wright, and C. Christiansen, Argonne National Laboratory Report ANL-6971, 1964, p 75.

(3) R. A. Jenkins, *J. Cell Biol.*, **23**, 46 (1964).

(4) M. M. P. Muir, *J. Chem. Soc. Trans.*, 1485 (1907).

(5) P. DuBois, *Compt. Rend.*, **200**, 1107 (1935).

(6) J. Pike and J. Hoste, *Anal. Chim. Acta*, **26**, 501 (1962).