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The Synthesis and Reactions of Some Carcinogenic *N*-(2-Phenanthryl)hydroxylamine Derivatives

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Abstract

The synthesis of 2-nitrophenanthrene from 9,10-dihydrophenanthrene is reported. Some 2-substituted phenanthrene and 9,10-dihydrophenanthrene hydroxylamine derivatives have been prepared and the structure of the rearrangement products determined.

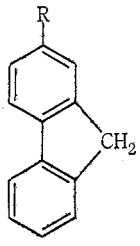
Ester conjugates of *N*-acyl-*N*-arylhydroxylamines have been implicated as the metabolites responsible for tumors produced in experimental animals after administration of certain aromatic amides.[1] These conjugates are highly labile and consequently *O*-acetate esters have been used extensively as models for *in vitro* studies and for biological testing.[1,2]

Most of these studies on the chemical carcinogenesis of *N*-arylhydroxylamines have made use of fluoren-2-ylhydroxylamine derivatives (1c) and (1d).[2] The ability of *N*-(2-phenanthryl)hydroxylamine derivatives (2c) and (2d) to induce sarcomas is significantly greater[2] than that of the corresponding fluorenyl compounds, but the difficulty of synthesis of 2-nitrophenanthrene (2a) which is the most convenient precursor of (2c) and (2d) has probably restricted the availability of the phenanthryl compounds. Direct nitration of phenanthrene gives a mixture of nitro compounds which cannot be easily separated.[3] An alternative multistep synthesis[4] of (2a) involves a Wagner–Meerwein ring expansion of the carbocyclic ring of 2-nitrofluoren-9-ylmethyl acetate which was prepared from fluorene; the overall yield was only 8%.[4] We have developed an efficient straightforward synthesis of 2-nitrophenanthrene (2a) by way of 2-nitro-9,10-dihydrophenanthrene (3a). Also (3a) has been converted into the hydroxylamine derivatives (3c) and (3d); these transformations in the dihydrophenanthrene series extend the number of polycyclic *N*-arylhydroxylamines available for carcinogenesis studies.

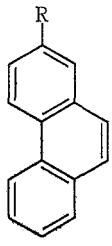
Solvolysis of *O*-esters of *N*-acyl-*N*-arylhydroxylamines leads to the formation of a nitrenium ion[5] which may undergo substitution either on the nitrogen atoms[6] or in

[1] Miller, J. A., *Cancer Res.*, 1970, **30**, 559.
[2] Miller, J. A., and Miller, E. C., *Progr. Exp. Tumor Res.*, 1969, **11**, 273.
[3] Dewar, M. J. S., and Warford, E. W. T., *J. Chem. Soc.*, 1956, 3570.
[4] Miller, E. C., Lotlikar, P. D., Pitot, H. C., Fletcher, T. L., and Miller, J. A., *Cancer Res.*, 1966, **26**, 2239.
[5] Gassman, P. G., and Campbell, G. A., *Chem. Commun.*, 1971, 1437.
[6] Kriek, E., Miller, J. A., Juhl, U., and Miller, E. C., *Biochemistry*, 1967, **6**, 177.

the aryl ring.[7,8] Electrophilic attack by such nitrenium ions on biological substrates *in vivo* has been suggested as the mechanism of carcinogenesis of these hydroxylamine derivatives.[1,2] A detoxifying mode of reaction for these esters is a thermal rearrangement resulting in migration of the ester group from nitrogen to the *ortho*-position on the aromatic ring. The thermal rearrangement of (2d) and (3d) has been investigated as more than one isomer is possible from these unsymmetrical hydroxylamine esters.



(1)



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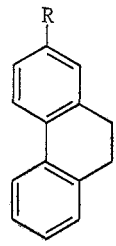


(3)

(a) R = NO_2
(b) R = NHOH
(c) R = N(Ac)OH
(d) R = N(Ac)OAc

Discussion

9,10-Dihydrophenanthrene was nitrated in acetic acid solution; whilst this reaction has been shown to give both 2- and 4-nitro-9,10-dihydrophenanthrenes[9] a slight modification of the workup procedure facilitated isolation of the 2-isomer (3a) in 60% yield. Attempted dehydrogenation of (3a) with metal catalysts or high potential quinones was unsuccessful, but (3a) was smoothly converted into (2a) in 83% yield by reaction with *N*-bromosuccinimide in refluxing carbon tetrachloride.

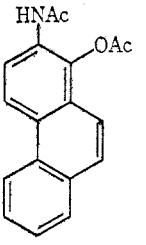
Reduction of 2-nitro-9,10-dihydrophenanthrene (3a) with hydrogen sulphide and ammonia according to the procedure of Lotlikar *et al.*[10] gave the hydroxylamine (3b) which was not characterized but acetylated directly with ketene. The hydroxamic acid (3c) was isolated by base extraction in 78% yield from the nitro compound. Acetylation of the sodium salt of (3c) in aqueous solution according to the method of Lotlikar *et al.*[7] gave the highly crystalline ester (3d) which showed infrared carbonyl absorptions at 1687 and 1787 cm^{-1} characteristic[11] of such *N,O*-diacetylhydroxylamine derivatives.

Conversion of (2a) into (2d) followed a similar procedure.[4,12]

Thermal rearrangement of *N,O*-diacetyl-*N*-phenanthrylhydroxylamine (2d) in nitrobenzene[1] gave a single crystalline product on workup and this showed an n.m.r. signal for the *O*-acetyl methyl group at δ 2·48. On the basis of the known substitution behaviour of phenanthrenes[13] this would be expected to be the 1-acetoxy isomer (4).

[7] Lotlikar, P. D., Scribner, J. D., Miller, J. A., and Miller, E. C., *Life Sci.* (Oxford), 1966, **5**, 1263.
[8] Calder, I. C., Creek, M. J., and Williams, P. J., *Chem. Biol. Interactions*, 1974, **8**, 87.
[9] Krueger, J. W., and Mosettig, E., *J. Org. Chem.*, 1938, **3**, 340.
[10] Lotlikar, P. D., Miller, E. C., Miller, J. A., and Margreth, A., *Cancer Res.*, 1965, **25**, 1743.
[11] Bartsch, H., Miller, J. A., and Miller, E. C., *Biochim. Biophys. Acta*, 1972, **273**, 40.
[12] Scribner, J. D., Miller, J. A., and Miller, E. C., *Cancer Res.*, 1970, **30**, 1570.
[13] Fieser, L. F., and Young, M. N., *J. Amer. Chem. Soc.*, 1931, **53**, 4120.

This isomer was prepared by acetylation of the known[14] hydrochloride salt of 2-amino-1-phenanthrol and shown to be identical with the rearrangement product from (2d). A 100-MHz n.m.r. study of the rearrangement mixture of the (2d) showed a small amount of a second isomeric *O*-acetyl methyl signal at δ 2·38 which was assigned to the 3-acetoxy isomer (5). This assignment was substantiated by the dehydrogenation experiment detailed below. Thus the rearrangement of the phenanthrene derivative (2d) is regioselective, giving at least 95% of the 1-acetoxy isomer (4). Similar regioselectivity has been observed in the Claisen rearrangement of 2-allyloxyphenanthrene derivatives to give 1-allyl-2-phenanthrol.[13]



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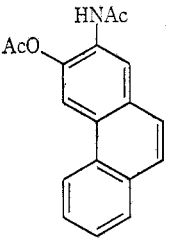

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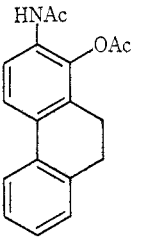

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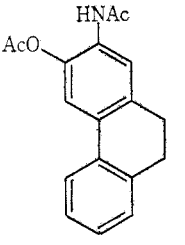

(7)

N,*O*-Diacetyl-*N*-[2-(9,10-dihydrophenanthryl)]hydroxylamine (3d) on thermal rearrangement gave a mixture of two isomeric products in a ratio of 3 : 2 as estimated from the ring *O*-acetate methyl signal intensities in the 100-MHz n.m.r. spectrum. These were isolated by fractional crystallization and separately identified as the 1- and 3-acetoxy isomers (6) and (7). The structure of these isomers follows from analysis of the n.m.r. spectra of each. The 3-acetoxy isomer (7) showed two singlets at δ 8·45 and 9·0, assigned to the 1- and 4-protons; in comparison an AB quartet δ 8·6 and 9·0 (J 8·5 Hz) was observed for the 3- and 4-protons in the 1-acetoxy isomer (6). In addition, the signal of the bridging methylene protons in (6) was very broad due to shielding by the 1-acetoxy group whereas these protons gave rise to a sharp singlet for the 3-acetoxy isomer (7).

The rearrangement products from the 9,10-dihydrophenanthrene derivative (3d) and phenanthrene derivative (2d) were interrelated by dehydrogenation of a mixture of (6) and (7) by reaction with dichlorodicyanoquinone in refluxing anisole to give (4) and (5). Whilst (4) and (5) could not be resolved by a number of chromatographic systems the isomers were well distinguished in the 100-MHz n.m.r. spectra, the *O*-acetate methyl signals appearing at δ 2·48 and 2·38 for the 1- and 3-acetoxy isomers respectively. Hence the presence of a small quantity of 3-acetoxy isomer (5) in the rearrangement mixture of the phenanthryl derivative (2d) could be demonstrated by this technique.

Experimental

Melting points were determined on a Kofler hot-stage microscope. The n.m.r. spectra were recorded on a Perkin–Elmer R12 and Varian HA100 spectrometer at 60 and 100 MHz respectively with tetramethylsilane as internal standard. Infrared spectra were determined as KBr discs on a

[14] Duvall, H. M., and Mosettig, E., *J. Amer. Chem. Soc.*, 1938, **60**, 2409.

Perkin–Elmer 457 spectrophotometer. Thin-layer chromatography was carried out on 0·25 mm plates using Merck silica gel GF adsorbent and 5% ethanol in chloroform as solvent.

Microanalyses were performed by the Australian Microanalytical Service, Melbourne.

2-Nitro-9,10-dihydrophenanthrene (3a)

9,10-Dihydrophenanthrene (60 g) was dissolved in acetic acid (300 ml) and fuming nitric acid (75 ml) added dropwise with stirring over 2 h, whilst maintaining the temperature at 15–20°C. When the addition was complete the mixture was cooled in an ice bath and the precipitate filtered. Recrystallization from ethanol yielded 2-nitro-9,10-dihydrophenanthrene (45 g, 60%), m.p. 81–82° (lit.[9] 81–82°).

2-Nitrophenanthrene (2a)

To a solution of 2-nitro-9,10-dihydrophenanthrene (5 g) in carbon tetrachloride (100 ml), *N*-bromosuccinimide (5 g) was added with stirring. The mixture was refluxed and irradiated with u.v. light until no further *N*-bromosuccinimide remained. The precipitated succinimide was removed by filtration and carbon tetrachloride evaporated under reduced pressure. Recrystallization of the residue from ethanol yielded 2-nitrophenanthrene (4·1 g, 83%) as yellow needles, m.p. 119–120°C (lit.[4] 119–120°) (Found: C, 75·4; H, 4·2; N, 6·1. Calc. for $C_{14}H_9NO_2$: C, 75·3; H, 4·1; N, 6·3%).

N-*Acetyl*-N-[*2-(9,10-dihydrophenanthryl)*]*hydroxylamine (2c)*

A solution of 2-nitro-9,10-dihydrophenanthrene (4 g, 1·78mM) in dimethylformamide (200 ml) and 95% ethanol (200 ml) at 10°C was saturated firstly with ammonia and then hydrogen sulphide, and stood overnight at room temperature.[10] The filtered reaction mixture was poured into water (1 l.) and then extracted with ether (3 × 100 ml). The extract was washed with water. After treating the ether solution with ketene (2mM) it was allowed to stand at room temperature for 30 min, at the end of which time t.l.c. showed no unacetylated hydroxylamine. The ether solution was extracted with 5% NaOH (3 × 50 ml) and the combined basic extracts acidified with 10% sulphuric acid at 5°C which precipitated the product. The *hydroxylamine* (2c) was filtered off and recrystallized from benzene–petrol (1 : 1) as colourless needles (3·5 g, 78%), m.p. 129° (Found: C, 76·2; H, 6·0; N, 5·6. $C_{16}H_{15}NO_2$ requires C, 75·9; H, 6·0; N, 5·5%). ν_{max} 3100 and 1613 cm^{-1}. The n.m.r. spectrum in $CDCl_3$ showed the following signals: δ 2·14 (s, 3H, acetyl methyl), 2·85 (s, 4H, bridging methylenes) and 7·2–7·8 (complex, 7H, aromatics).

N,O-*Diacetyl*-N-[*2-(9,10-dihydrophenanthryl)*]*hydroxylamine (3d)*

The hydroxylamine (3c) (300 mg) was dissolved in 2% NaOH solution (3·0 ml) at room temperature and to this solution was added acetic anhydride (0·15 ml). After stirring for 30 min, the reaction mixture was extracted with chloroform and the solution washed with water, dried and evaporated. Recrystallization of the residue from benzene–petrol (1 : 1) gave the *hydroxylamine* (3d) as white plates (200 mg, 57%), m.p. 88° (Found: C, 73·3; H, 5·8; N, 4·6. $C_{18}H_{17}NO_3$ requires C, 73·2; H, 5·8; N, 4·7%). ν_{max} 1787 and 1687 cm^{-1}. The n.m.r. spectrum in $CDCl_3$ showed the following signals: δ 2·05 (s, 3H, *N*-acetyl methyl), 2·15 (s, 3H, *O*-acetyl methyl), 2·82 (s, 4H, bridging methylenes), 7·2 and 7·6 (complex, 7H, aromatics).

Thermal Rearrangement of N,O-*Diacetyl*-N-[*2-(9,10-dihydrophenanthryl)*]*hydroxylamine (3d)*

N,O-Diacetyl-*N*-[2-(9,10-dihydrophenanthryl)]hydroxylamine (3d) (1·0 g) was heated at 120°C for 2 h. The sample was crystallized from benzene–petrol to yield a mixture of rearranged acetates. Fractional recrystallization from benzene, followed by sublimation at 150°/0·1 mm yielded *2-acetylamino-9,10-dihydro-1-phenanthryl acetate* as white needles, m.p. 196–197°C (Found: C, 73·2; H, 5·9. $C_{18}H_{17}NO_3$ requires C, 73·2; H, 5·8%). Fractional recrystallization of the combined mother liquors from carbon tetrachloride yielded *2-acetylamino-9,10-dihydro-3-phenanthryl acetate* as white needles, m.p. 179–180°C (Found: C, 73·3; H, 5·8. $C_{18}H_{17}NO_3$ requires C, 73·2; H, 5·8%).

Thermal Rearrangement of N,O-*Diacetyl*-N-(*2-phenanthryl*)*hydroxylamine (2d)*

A solution of (2d) (200 mg) in nitrobenzene (2·0 ml) was heated at 100°C for 6 days. The solvent was removed by vacuum distillation and the residue crystallized from chloroform–benzene (1 : 1)

to give *2-acetylamino-1-phenanthryl acetate* (4) (100 mg), m.p. 225° (EtOH) (Found: C, 73·8; H, 5·3; N, 4·6. $C_{18}H_{15}NO_3$ requires C, 73·7; H, 5·1; N, 4·8%). ν_{max} 1690 and 1725 cm^{-1}. The n.m.r. spectrum in $(CD_3)_2SO$ showed the following signals: δ 2·11 (3H, s, *N*-acetyl methyl), 2·48 (3H, s, *O*-acetyl methyl), 7·8 and 8·7 (8H, multiplet, aromatics).

2-Acetylaminophenanthryl Acetates (4) and (5)

A mixture of (6) and (7) (50 mg) from thermal rearrangement of (3) was heated with dichlorodicyanoquinone (50 mg) in refluxing anisole (2 ml) for 1·5 h. After dilution with chloroform the reaction mixture was shaken with 1N NaOH until no further colour was extracted into the base. The organic solution was then washed with water and dried; the solvent was removed by distillation under vacuum. T.l.c. of the residue showed the product to be identical with (4) synthesized below. The n.m.r. spectrum in $(CD_3)_2SO$ showed a broad *N*-acetyl methyl signal at δ 2·1 and two *O*-acetyl methyl signals at δ 2·38 and 2·48.

2-Acetylamino-1-phenanthryl Acetate (4)

1-Oxo-1,2,3,4-tetrahydrophenanthrene was prepared from naphthalene by a standard procedure,[15] m.p. 95–96° (lit.[15] 95–96°).

To a solution of 1-oxo-1,2,3,4-tetrahydrophenanthrene (1·5 g) in carbon tetrachloride (25 ml), *N*-bromosuccinimide (1·5 g) was added with stirring. The mixture was refluxed and irradiated with u.v. light until no further *N*-bromosuccinimide remained. The precipitated succinimide was removed by filtration and the carbon tetrachloride solution extracted with aqueous sodium hydroxide (2 × 10 ml, 5%). The base extract was acidified and extracted with methylene chloride. The extract was dried ($MgSO_4$) and evaporated to dryness. Sublimation of the residue yielded 1-phenanthrol (1·1 g), m.p. 153–154° (lit.[16] 153–154°).

1-Phenanthrol was converted into the hydrochloride salt of 2-amino-1-phenanthrol by following the synthesis of Duvall and Mosettig.[14] The amine salt (50 mg) was acetylated by refluxing in acetic anhydride (5 ml) with sodium acetate (100 mg) for 2 h. The reaction mixture was poured into water, extracted with chloroform and the extract evaporated. Recrystallization of the residue from 95% ethanol gave (4), 50 mg (83%), m.p. 225°. This product showed identical t.l.c. and i.r. as well as undepressed mixed m.p. with the product isolated from thermal rearrangement of (2d) above.

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[15] Haworth, R. D., *J. Chem. Soc.*, 1932, 1125.
[16] Mosettig, E., and Duvall, H. M., *J. Amer. Chem. Soc.*, 1937, **59**, 367.