Synthesis of Intercalating and Carcinogenic Nitroxide Spin Labels

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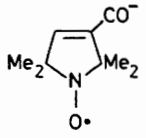
Summary Several new and useful nitroxide spin labels have been prepared for studies in carcinogen binding to cellular constituents using simple organic reactions.

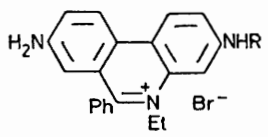
THE use of nitroxide spin labels to probe the macromolecular structure in a variety of biological systems has been well established[1]. It has been suggested that intercalation is the

dominant mode of binding for most aromatic amine hydrocarbons with native DNA. This mechanism has been verified and is discussed in detail elsewhere.[2] We now describe the specific compounds used as labels in these studies and their synthesis.

Spin-labelled ethidium bromide (I) was prepared by adding dropwise to a two-fold molar excess of ethidium bromide in dichloromethane containing pyridine, a solution of 3-chloroformyl-2,2,5,5-tetramethylpyrrolin-1-oxyl, prepared according to the method of Rozantsev,[3] in dichloromethane. Workup (including anion exchange) followed by preparative t.l.c. on silica gel with ethanol–chloroform as eluent gave compound (I) in low yield; orange solid, m.p.



(A)



(I)

228—233 °C† (decomp.; progressive darkening from *ca.* 70 °C; ν_{max} 3400, 1675, 1610, 1510, 1280, 740, and 695 cm^{-1}; the mass spectrum was not useful for characterisation owing to extensive pyrolysis. A quantitative e.s.r. absorption measurement of the nitroxide triplet of (I) was consistent with its calculated molecular weight. The attachment of the pyrrolin-3-ylcarbonyl group at the 2-amino-group rather than the 7-amino-group was deduced from space filling models which indicated appreciable steric blocking from the phenyl ring at the 7-amino group. Accommodation of the pyrrolin-3-ylcarbonyl group at the 7-amino-group would necessitate distortion of the planar phenanthridine ring to a destablized non-planar conformation. Attachment at the 2-amino-group is easily accommodated sterically.

A spin labelled aniline derivative, 3-(anilinocarbonyl)-2,2,5,5-tetramethylpyrrolin-1-oxyl, was prepared by the dropwise addition of an ether solution of 3-chloroformyl-2,2,5,5-tetramethylpyrrolin-1-oxyl to a twofold excess of aniline in ether. Workup followed by crystallization from chloroform–hexane yielded the desired compound, yellow solid, m.p. 178—179 °C, M^+ 259; ν_{max} 3300, 2990, 1660, 1600, 1540, 1445, and 750 cm^{-1}.

Further spin-labelled derivatives containing the substituted pyrrolin-1-oxyl group (A) substituted on the amino-nitrogen atom in the following compounds were prepared analogously: 1-aminonaphthalene, spin-labelled derivative, pink needles from chloroform–hexane, m.p. 86—89 °C (decomp.); M^+ 309; ν_{max} 3250, 2980, 1655, 1615, 1500, 1350, 1290, 790, and 750 cm^{-1}; 2-aminoanthracene, 6-aminochrysene, 2- aminofluorene, spin-labelled derivative m.p. 114—119 °C (decomp.), and 3-nitroaniline, spin-labelled derivative, yellow crystals from ether–methanol, m.p. 227—229 °C (decomp.), M^+ 304; ν_{max} 3340, 2980, 2940, 1675, 1530, and 1340 cm^{-1}.

A spin-labelled derivative of 3,5-dinitrobenzenzoic acid, 4-(3,5-dinitrobenzenoylamino)-2,2,6,6-tetramethylpiperidin-1-oxyl, was prepared by mixing 3,5-dinitrobenzenoyl chloride (prepared from the acid and thionyl chloride in chloroform) with a twofold molar excess of 4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl in chloroform. Workup followed by crystallization from ethanol yielded the desired compound, orange iridescent needles, m.p. 226—229 °C, ν_{max} 1660, 1530, and 1350 cm^{-1}.

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† All compounds were homogeneous on t.l.c. M.p.s were recorded on a Fisher–Johns apparatus and are uncorrected. Only major i.r. absorption bands (KBr discs) are given.

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