Spin Label Synthesis using *NN'*-Thionyldiimidazole Coupling Reagent

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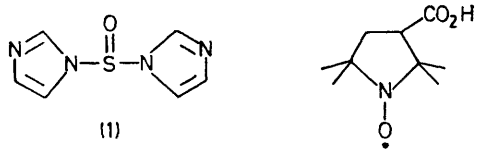
Summary Spin-labelled esters of both cortisol and testosterone have been synthesized using the coupling reagent *NN'*-thionyldiimidazole (**1**); use of more conventional coupling or dehydrating reagents failed to give these spin labels, suggesting that (**1**) may be useful in the esterification of other hindered, relatively unreactive alcohols.

THE application of e.s.r. spectroscopic methods to the examination of biological systems is dependent upon the synthesis of the appropriate stable free radical probes or reporter groups (spin labels[1]). One biological system of considerable importance is the binding of cortisol and related steroidal hormones to corticosteroid binding globulin in blood serum. We now report the successful synthesis of spin-labelled esters of cortisol and testosterone using *NN'*-thionyldiimidazole (**1**); several attempts using other coupling and dehydrating reagents were unsuccessful. These results suggest that (**1**) should be used as a coupling reagent in the synthesis of other ester spin labels (and esters in general), especially in cases involving hindered, relatively unreactive systems.

Di-t-alkylnitroxides[1] are the most widely used spin labels since they are among the most stable free radicals



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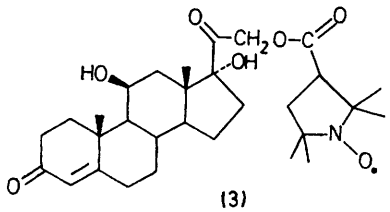
(2)



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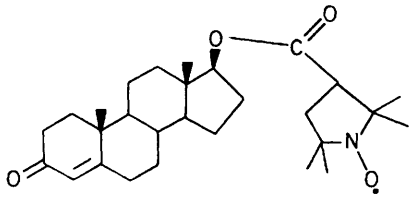

(4)

known. It was anticipated that the 3-carboxy-2,2,5,5-tetramethylpyrrolidin-1-yloxyl free radical[2] (**2**)† could be used to synthesize spin-labelled derivatives of cortisol and testosterone *via* esterification at the (most reactive) C(**21**) and C(17β) hydroxyl groups, respectively. The di-t-alkylnitroxide group, however, is destroyed[1] irreversibly at temperatures above *ca.* 70° and in the presence of strongly acidic conditions, thus necessitating relatively mild reaction conditions for the proposed esterification.

Use under mild conditions of the esterification reagents dicyclohexylcarbodiimide,[3] trifluoroacetic anhydride,[4] and trifluoroacetic anhydride containing added pyridine, however, failed to afford the required spin-labelled steroidal esters.

In sharp contrast, the use of *NN'*-thionyldiimidazole[5] (**1**) as the coupling reagent, which to our knowledge has not been used in previous spin label syntheses, gave the C(**21**) cortisol ester (**3**) in 16% yield‡ and the C(17β) testosterone ester (**4**) in 8% yield‡ after reaction periods at ambient temperature of 24 h and 45 h, respectively. In each case, the paramagnetic di-t-alkyl nitroxide group was found by e.s.r. to be completely intact.

The cortisol spin-labelled ester (**3**) was recrystallized from acetone as pale yellow crystals; m.p. 225—226; i.r. ($CHCl_3$) 3700—3200, 1737, 1727, 1660 and 1100 cm^{-1}. The testosterone spin-labelled ester (**4**) was recrystallized from acetone–ether as pale yellow needles; m.p. 206·5—207·5°; i.r. ($CHCl_3$) 1725, 1662 and 1100 cm^{-1}. Both (**3**) and (**4**) afforded satisfactory elemental analyses.

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† Attempts to prepare the acid chloride of (**2**) without destruction of the nitroxide group were unsuccessful.

‡ Yield of analytically pure isolated material.

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