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Syntheses of Haptens Related to the Benzenoid and Indole Portions of Sporidesmin A; ^{13}C N.M.R. Spectra of Indole Derivatives

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Abstract

The syntheses of 2-amino-5-chloro-3,4-dimethoxybenzyl alcohol (2) and 5-chloro-6,7-dimethoxy-*N*-methyl-1*H*-indol-3-yloxoacetic acid (22), potential haptens related to the benzenoid and indole portions of the mycotoxin sporidesmin, are described. ^{13}C n.m.r. assignments for the latter and ten related indole compounds are given.

Introduction

Sporidesmin A (1), the main toxin responsible for causing facial eczema in cattle and sheep,[1] has been structurally analysed by X-ray crystallography[2] and recently synthesized in racemic form.[3] In an attempt to produce an immune response to the compound in experimental animals, Jonas and Ronaldson,[4] after opening the disulfide linkage, condensed the toxin with poly-L-lysine, and found modest production of antibodies in response to injection of this artificial antigen. The present report describes synthetic work undertaken in a complementary approach which involves the application of fully synthetic haptens based on the benzenoid and indole parts of sporidesmin. Observations made in the course of preparing compounds (2) and (22) are recorded together with ^{13}C n.m.r. data on indole compounds used in the study.

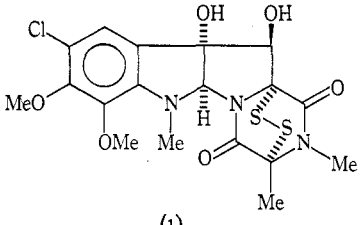
Results and Discussion

The amino alcohol (2) was prepared by the reduction with lithium aluminium hydride of the known amino ester (3) which was synthesized in seven steps from vanillin via the triacetate (4) and the aldehyde (5). Oxidation of this aldehyde, followed by dimethylation, gave the nitro ester (6) and reduction of this with stannous chloride in ethanolic hydrogen chloride then yielded the amino ester (3). Previously,[5] compound (3) had been produced from vanillin in an eleven-step process involving nitration of the chloro ester (7).

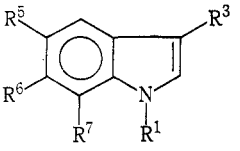
An alternative approach to the synthesis of the hapten (2) was not successful. This required catalytic hydrogenation of 5-chloro-2-nitroveratraldehyde (8), obtained by

[1] Ronaldson, J. W., *Aust. J. Chem.*, 1976, **29**, 2307, and earlier parts of this series.
[2] Fridrichsons, J., and Mathieson, A. M., *Tetrahedron Lett.*, 1962, 1265; *Acta Crystallogr.*, 1965, **18**, 1043; Beecham, A. F., Fridrichsons, J., and Mathieson, A. M., *Tetrahedron Lett.*, 1966, 3131.
[3] Kishi, Y., Nakatsuka, S., Fukuyama, T., and Havel, M., *J. Am. Chem. Soc.*, 1973, **95**, 6493.
[4] Jonas, W. E., and Ronaldson, J. W., *N.Z. Vet. J.*, 1974, **22**, 111.
[5] Hodges, R., and Taylor, A., *J. Chem. Soc.*, 1964, 4310.

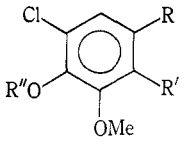
methylation of the 4-hydroxy analogue (5), but the nitro group was preferentially reduced during the hydrogenation, and the amino aldehyde produced then underwent cyclic trimerization.[6] Although this side reaction was precluded by pre-reduction of the aldehyde (8) with sodium borohydride, the product (9) gave the hapten (2) in only low yield when the nitro group was reduced with iron in hydrochloric acid.



(1)



	R^1	R^3	R^5	R^6	R^7
(12)	H	H	H	H	H
(13)	Me	H	H	H	H
(14)	H	CHO	H	H	H
(15)	Me	CHO	H	H	H
(16)	Me	$COCO_2H$	H	H	H
(17)	H	H	H	MeO	MeO
(18)	Me	H	H	MeO	MeO
(19)	H	H	Cl	MeO	MeO
(20)	Me	H	Cl	MeO	MeO
(21)	Me	$COCO_2H$	H	MeO	MeO
(22)	Me	$COCO_2H$	Cl	MeO	MeO
(23)	Me	$CH{=}N^+(Me_2)Cl^-$	H	H	H
(24)	Me	$CH{=}N^+(Me_2)I^-$	H	H	H
(25)	Me	$CH{=}C(CO_2Me)_2$	H	H	H
(26)	Me	$CH{=}C(CO_2Et)_2$	H	H	H



	R	R′	R″
(2)	CH_2OH	NH_2	Me
(3)	CO_2Me	NH_2	Me
(4)	$CH(OAc)_2$	H	Ac
(5)	CHO	NO_2	H
(6)	CO_2Me	NO_2	Me
(7)	CO_2Me	H	Ac
(8)	CHO	NO_2	Me
(9)	CH_2OH	NO_2	Me
(10)	$CH(OH)CH_2NO_2$	NO_2	Me
(11)	$HC{=}CHNO_2$	NO_2	Me

The required hapten (2) was an oil which was characterized by conversion into the following crystalline derivatives: the *N,O*-dibenzoate, the 2-hydroxynaphthalenylazo dye and the 2-phenyloxazaborine. This last derivative was prepared by condensation with phenylboronic acid, and was used directly for the condensation of the hapten to biopolymers by way of the azo linkage.[7]

A significant feature of mono-nitration reactions in this series of compounds is the apparent relationship between the nature of the substituent at C4 and the site of the main substitution. 3-Methoxybenzaldehyde thus nitrates mainly at C2[8] as do its 4-acetoxy[9] and 4-acetoxy-5-chloro[10] derivatives, whereas the 4-methoxy compound (veratraldehyde) gives mainly the 6-nitro product.[11] 5-Chloroveratraldehyde, if

[6] Cummings, S. C., and Busch, D. H., *Inorg. Chem.*, 1971, **10**, 1220.
[7] Jonas, W. E., and Erasmuson, A. F., *N.Z. Vet. J.*, 1977, **25**, 161.
[8] Hodgson, H. H., and Beard, H. G., *J. Chem. Soc.*, 1926, 147.
[9] Raiford, L. C., and Floyd, D. E., *J. Org. Chem.*, 1943, **8**, 358.
[10] Raiford, L. C., and Lichty, J. G., *J. Am. Chem. Soc.*, 1930, **52**, 4576.
[11] Fetscher, C. A., *Org. Synth.*, 1963, Coll. Vol. IV, 735.

treated with concentrated nitric acid, is now found to react similarly, but when this compound was nitrated with fuming nitric acid, the main product was a dinitro compound (m.p. 125–126°), devoid of carbonyl groups, which is assumed to be 3-chloro-1,2-dimethoxy-4,5-dinitrobenzene by analogy with previous findings.[12] Raiford and Floyd,[9] using these conditions, reported a product from this reaction with m.p. 122–123° and containing 13·7% chlorine, which they described as 5-chloro-6-nitroveratraldehyde (Cl, 14·4%). However, this compound, which has recently been characterized by ^{1}H and ^{13}C n.m.r. methods, is reported[13] to have m.p. 135–136°, and it therefore appears probable that Raiford and Floyd also obtained the dinitro derivative (Cl, 13·7%).

The route selected for the preparation of the indole hapten (22) involved standard base-catalysed reaction between the aldehyde (8) and nitromethane to give the adduct (10) from which the crystalline (*E*)-styrene (11) was obtained in high yield. Reductive ring closure with iron in acetic acid[14] gave the indole (19), methylation[15] of which afforded the *N*-methyl derivative (20), and from this, the hapten (22) was obtained following treatment with oxalyl chloride and hydrolysis. Ethanolysis of the solid product obtained by use of oxalyl chloride gave the ethyl ester of the acid (22), showing that the intermediate was the oxoacetyl chloride. Kishi *et al.*[3] had previously prepared the *N*-methylindole (20) by reduction of the corresponding oxoindole.

Additionally, a series of dechloro analogues [(14)–(18), (21)] were prepared since it has been shown that immunological responses to complexed haptens of this series are dependent on the presence of the chlorine atoms.[16]

In model experiments having as their objective the preparation of the pyrroloindole ring system of sporidesmin, *N*-methylindole (13) was treated with phosphoryl chloride in *N*,*N*-dimethylformamide (Vilsmeier–Haack reaction[17]) and gave the imonium salt (23) in quantitative yield. On reaction with sodium iodide in methanol, anion exchange occurred to give the corresponding iodide (24) and on hydrolysis *N*-methylindole-3-carbaldehyde (15) was formed. Treated with dimethyl malonate in methanol containing sodium methoxide, the chloride (23) gave the alkene (25) [and the diethyl analogue (26) was prepared similarly], but no condensation could be detected when the anion derived from diethyl *N*-acetylaminomalonate was used.

^{13}C N.M.R. Data

The present data complement those reported recently[1] for sporidesmin itself. It is now possible to analyse the spectrum of the indole portion of the toxin unencumbered by the dioxopiperazine moiety, and it is therefore expected that this may be of utility in characterizing the structures of mycotoxins related to sporidesmin, as well as providing additional data for substituent effects in the indole ring system. In Table 1 chemical shift data for 11 variously substituted indoles are given. All were obtained for $CDCl_3$ solutions, except in the case of compound (14) which was insoluble in this solvent. For compound (20) the spectrum was also determined for a

[12] Heacock, R. A., and Hutzinger, O., *J. Chem. Soc.*, 1963, 3574.
[13] Erasmuson, A. F., Ferrier, R. J., Franca, N. C., Gottlieb, H. E., and Wenkert, E., *J. Chem. Soc., Perkin Trans. 1*, 1977, 492.
[14] Julia, M., Manoury, P., and Voillaume, C., *Bull. Soc. Chim. Fr.*, 1965, 1417.
[15] Potts, K. T., and Saxton, J. E., *Org. Synth.*, 1960, **40**, 68.
[16] Erasmuson, A. F., and Jonas, W. E., unpublished data.
[17] Seshadri, S., *J. Sci. Ind. Res.*, 1973, **32**, 128.

[D_6]acetone solution to allow comparison with the published spectrum of sporidesmin.[1] The assignments for indole (12) and *N*-methylindole (13) are those given in ref.[18], while the spectrum of indole-3-carbaldehyde (14) has been reported in ref.[19] For the remaining compounds, assignments for the ring carbons were made by considering the effects observed on introduction of the various substituents into the parent indoles (12) and (13), as described below. Routine use was made of the observation that, under the spectral acquisition conditions employed, the resonances of non-protonated carbons were always of considerably lower intensity than the resonances of protonated carbons.

Table 1. ^{13}C n.m.r. shielding data for $CDCl_3$ solutions of indoles

All shielding values are in ppm, ±0·1, relative to tetramethylsilane (0 ppm)

Cpd	C2	C3	C4	C5	C6	C7	C3a	C7a	R^1	R^3	R^6	R^7
(12)	124·4	102·0	120·6	121·8	119·7	111·2	127·6	135·6	—	—	—	—
(13)	128·6	100·8	120·8	121·4	119·2	109·1	128·6	136·7	32·2	—	—	—
(14)[A]	139·1	118·8	124·2	122·9	121·5	113·1	124·7	137·7	—	186·0	—	—
(15)	139·8	117·6	123·7	122·6	121·5	109·9	124·9	137·7	33·1	184·1	—	—
(16)	143·2	109·9	124·7	124·2	122·7	110·3	123·0	139·2	34·0	161·0, 184·5	—	—
(17)	124·3	102·5	115·6	108·7	146·9	134·6	124·8	130·5	—	—	57·5	60·7
(18)	129·9	100·9	115·8	108·6	147·8	136·2	126·3	130·3	35·2	—	57·4	61·5
(19)	125·3	102·5	116·0	121·1	142·8	139·2	125·6	128·9	—	—	61·4	60·9
(20)	131·1	100·8	116·1	120·7	143·8	140·7	127·0	128·6	35·4	—	61·2	61·8
(20)[B]	132·3	101·3	116·6	120·9	144·4	141·6	128·0	129·5	35·5	—	61·2	62·1
(21)	144·8	110·7	117·6	111·7	149·9	136·4	123·6	130·8	37·2	161·2, 174·9	57·0	61·8
(22)	144·7	110·2	118·0	125·3	146·1	141·4	126·1	129·3	37·3	160·8, 175·3	61·2	62·0

[A] [D_6]Dimethyl sulfoxide solvent.
[B] [D_6]Acetone solvent.

The assignments for the resonances in the spectrum of compound (15) followed by taking the effects observed when the 3-formyl group is introduced into indole (12), and adding these to the observed shieldings for *N*-methylindole (13). The agreement of calculated and observed shieldings was very good, considering that the spectra of compounds (12) and (14) were obtained in different solvents. The spectrum of the acid (16) was assigned by direct comparison with that of aldehyde (15). The effect of introducing methoxyl groups at carbons 6 and 7 as in compound (17) could be estimated from a comparison of the spectra of 1,2-dimethoxybenzene and benzene. The resultant changes, when applied to the shieldings for indole (12), gave calculated values for the dimethoxy derivative (17) in fair agreement with those observed for carbons 4–7, while marked deviations were found, not surprisingly, for carbons 3a and 7a. However, the assignments shown for carbons 3a and 7a in compound (17) are considered to be justified from a consideration of the effects of other substituent changes on these values. Further substitution, of a methyl on the nitrogen atom to give compound (18), resulted in changes analogous to those observed in changing from indole (12) to its *N*-methyl derivative (13), except that the upfield shift observed in the latter case for carbon 7 has become a downfield shift for carbon 7 when the methoxyl group is

[18] Parker, R. G., and Roberts, J. D., *J. Org. Chem.*, 1970, **35**, 996.
[19] Rosenberg, E., Williamson, K. L., and Roberts, J. D., *Org. Magn. Reson.*, 1976, **8**, 117.

present. This is a consequence of the upfield γ-effect shift for compound (13) being replaced by a δ-type interaction between the *N*-methyl and 7-methoxyl groups in compound (18). Such an interaction usually causes downfield shifts for the carbon bearing the substituent.[20]

The assignments for the 5-chloro substituted compounds (19) and (20) followed from application of the known substituent effects of chlorine in a benzene ring[21] to the shieldings for compounds (17) and (18), respectively. For carbons 3a, 4 and 7a, the agreement between calculated and observed shieldings was very good, although deviations occurred for the substituted carbons 5, 6 and 7, presumably as a consequence of the steric interactions between the substituents on these carbons.[13]

Finally, the assignments for the compounds (21) and (22) were made by noting the effects resulting from introduction of the oxoacetic acid group into *N*-methylindole (13) and applying these to the shieldings for compounds (18) and (20), respectively. The resultant calculated shieldings, when compared with the observed shieldings, allow the assignments, as shown in Table 1, to be made. A further check on these assignments can be made by noting that the effects of introducing a chlorine substituent at carbon 5 of (21) are very similar to those found in changing from compound (18) to (20).

Substituent carbon resonances were assigned readily, since the *N*-methyl carbon always gave an absorption at 32–37 ppm, while the oxoacetic acid group showed resonances for the carboxyl carbon at $161{\cdot}0 \pm 0{\cdot}2$ ppm, and for the oxo carbon at $175{\cdot}1 \pm 0{\cdot}2$ ppm in the dimethoxy compounds (21) and (22) but $184{\cdot}5$ in compound (16). The formyl carbon for compounds (14) and (15) appeared at *c.* 185 ppm. The methoxyl carbon resonances for compounds (17)–(22) could be assigned to the carbon 6 or 7 substituents by noting that introduction of chlorine at carbon 5 produced a downfield shift of *c.* 4 ppm on one resonance, but had very little effect on the other; this thus suggests that the former arose from the 6′-methoxyl carbon. Support for this assignment was provided by the observation that the resonance less affected by introduction of the chlorine was more affected on introduction of a methyl group on the nitrogen atom. A downfield shift of *c.* $0{\cdot}8$ ppm on the methoxyl resonance thus assigned to carbon 7′ ensued.

Experimental

The ^{13}C n.m.r. spectra were recorded in the repetitive pulse FT mode on a Varian CFT-20 spectrometer for $CDCl_3$ solutions unless otherwise stated. Typically, spectral widths of 4000 Hz were examined with pulse intervals of 1–4 s and flip angles of 30°.

Methyl 2-Amino-5-chloro-3,4-dimethoxybenzoate (3)

Recrystallized 4-acetoxy-3-chloro-5-methoxybenzylidene diacetate (4) (40 g), prepared in 60% yield from vanillin via 5-chlorovanillin according to the method of Raiford and Lichty,[10] was added slowly with stirring to fuming nitric acid (100 ml) with cooling so that the mixture was kept below 0°. After the addition was complete (15 min), stirring was continued for a further 10 min, and the red solution was poured onto crushed ice (500 g) to give a solid. After washing, drying and recrystallization from benzene, the 6-chloro-4-formyl-2-methoxy-3-nitrophenyl acetate (26 g, 78%) had m.p. 110–111° (lit.[10] 112°); recrystallized from ethanol it gave a monoethanolate, m.p. 94–95° (lit.[10] 95–96°). Hydrolysis with sodium hydroxide in aqueous ethanol gave the corresponding phenol (5) [93%, m.p. 137–138° (from chloroform) (lit.[10] 137°)].

[20] Stothers, J. B., and Tan, C. T., *Can. J. Chem.*, 1976, **54**, 917.
[21] Stothers, J. B., 'Carbon-13 N.M.R. Spectroscopy' (Academic Press: New York 1972).

Freshly prepared silver oxide (from silver nitrate, 3·57 g) was suspended in water (25 ml), sodium hydroxide (2 g) was added and the mixture was heated to 50°. The above chloronitrovanillin (2·31 g) was added, the mixture was stirred for 15 min, filtered and the residues were washed with hot water. The combined filtrate and washings were then treated with sulfur dioxide, hydrochloric acid (60 ml, 5 M) was added and the solution was left at 4° for 16 h after which the precipitated 5-chloro-4-hydroxy-3-methoxy-2-nitrobenzoic acid (2·47 g, 100%) was removed, washed and dried. Recrystallized from chloroform/methanol it had m.p. 192–194° (lit.[5] 197–199°).

The hydroxybenzoic acid (20 g) was then added to a mixture of dimethyl sulfate (20 ml), acetonitrile (100 ml) and anhydrous potassium carbonate (35 g), and heated under reflux for 10 min. The mixture was cooled, ammonium hydroxide was added slowly until effervescence ceased, followed by acetic acid (25 ml), and the solution was poured into cooled water. After stirring for 15 min, the precipitated trimethyl compound (6) (20 g, 90%) was removed and dried. Recrystallized from ethanol it had m.p. 62–64° (lit.[5] 64°). This methyl 5-chloro-3,4-dimethoxy-2-nitrobenzoate (20 g) was dissolved in ethanol (500 ml), hydrochloric acid (250 ml, 2 M) was added and the mixture was heated to boiling. Stannous chloride solution [prepared by heating stannous chloride (20 g) and granulated tin (20 g) in hydrochloric acid (200 ml, 10·6 M) for 2 h] was slowly added, and the mixture was heated under reflux for 1·5 h. Removal, under vacuum, of the majority of the ethanol caused the amine hydrochloride [16 g, 78%, m.p. 200°C (dec.)] to precipitate. Dissolution of the salt in dilute aqueous sodium hydroxide and extraction with chloroform (100 ml) gave a solution from which, after drying, the amine (3) (10·9 g, 61%) was obtained after addition of light petroleum. It had m.p. 37–39° (lit.[5] 37–39°).

2-Amino-5-chloro-3,4-dimethoxybenzyl Alcohol (2)

The amino ester (3) (5 g) in ether (35 ml) was added to a stirred suspension of lithium aluminium hydride (1·5 g) in ether (25 ml) at a rate sufficient to maintain gentle reflux. After the addition (30 min) and further stirring for 15 min, water (2 ml), aqueous sodium hydroxide (2 ml, 15%) and further water (6 ml) were added successively, the ether solution was filtered, the residue washed with ether and the combined ether solutions were dried and the solvent was removed. The resulting oil *amino alcohol* (2) (4·3 g, 97%) was homogeneous by t.l.c. and ^{1}H n.m.r. examination, and was converted into three crystalline derivatives.

2-Benzoylamino-5-chloro-3,4-dimethoxybenzyl Benzoate

The amino alcohol (2) (0·22 g) was shaken with small amounts of benzoyl chloride and aqueous sodium hydroxide to give a solid. Recrystallized from methanol the *dibenzoyl derivative* (0·29 g, 68%) had m.p. 148–149° (Found: C, 64·5; H, 4·9; Cl, 8·3; N, 3·3. $C_{23}H_{20}ClNO_5$ requires C, 64·9; H, 4·7; Cl, 8·3; N, 3·3%).

*6-Chloro-7,8-dimethoxy-2-phenyl-1,2-dihydro-4*H*-3,1,2-benzoxazaborine*

The amino alcohol (2) (5 g) was heated under reflux with triphenylboroxole (3 g) in benzene (50 ml) with removal of the liberated water. Removal of the solvent left the crystalline cyclic derivative; recrystallized from propan-2-ol the *oxazaborine* (5·65 g, 81%) had m.p. 123–125° (Found: C, 59·2; H, 5·1; Cl, 12·0; N, 4·7. $C_{15}H_{15}BClNO_3$ requires C, 59·4; H, 5·0; Cl, 11·7; N, 4·6%).

5-Chloro-2-(2-hydroxynaphthalen-1-ylazo)-3,4-dimethoxybenzyl Alcohol

The benzoxazaborine (0·15 g) was dissolved in methanol (8 ml) containing hydrochloric acid (2 ml, conc.) and cooled in an ice bath. Sodium nitrite (0·055 g) in cooled water (5 ml) was added followed by 2-naphthol (0·078 g) in cooled aqueous sodium hydroxide (15 ml, 10%). The red precipitate was removed and recrystallized from methanol to give the *azo dye* (0·13 g, 70%), m.p. 185–186° (Found: C, 61·0; H, 4·7; Cl, 9·8; N, 7·6. $C_{19}H_{17}ClN_2O_4$ requires C, 61·2; H, 4·6; Cl, 9·5; N, 7·5%).

Nitration of 3-Chloro-4,5-dimethoxybenzaldehyde

(A) *With concentrated nitric acid.*—The aldehyde (2 g) was heated in nitric acid (*d* 1·4) until effervescence began; the solution was then immediately removed from the heat, left for 15 min and

poured into ice water to give the 2-nitro derivative (2·16 g, 88%), m.p. 134–135°, identical with the compound made in 81% yield by methylation with dimethyl sulfate of 3-chloro-4-hydroxy-5-methoxy-2-nitrobenzaldehyde. Both of these compounds were characterized by ^{13}C n.m.r. methods;[13] the *dimethoxy compound* was analysed (Found: C, 44·3; H, 3·3; Cl, 14·5; N, 5·7. $C_9H_8ClNO_5$ requires C, 44·1; H, 3·3; Cl, 14·5; N, 5·7%).

(B) *With fuming nitric acid.*—The aldehyde (0·40 g) was added to fuming nitric acid (10 ml) and, after 5 min, poured onto crushed ice. The precipitate was washed, dried and recrystallized from methanol to give *3-chloro-1,2-dimethoxy-4,5-dinitrobenzene* (0·36 g, 69%), m.p. 125–126° (Found: C, 36·7; H, 2·8; Cl, 13·7; N, 10·7. $C_8H_7ClN_2O_6$ requires C, 36·6; H, 2·7; Cl, 13·5; N, 10·7%).

5-Chloro-3,4-dimethoxy-2-nitrobenzaldehyde (8)

5-Chloro-2-nitrovanillin (5) (10 g) was methylated by heating under reflux in acetonitrile (20 ml) and dimethyl sulfate (5 ml) in the presence of potassium carbonate (10 g) for 10 min. The mixture was poured into aqueous ammonium hydroxide (40 ml, 2 M) and the solid which was obtained (10·1 g, 95%) was removed, dried and recrystallized from carbon tetrachloride/light petroleum to give the dimethoxy compound, m.p. 83–84° (lit.[8] 51–52°) (Found: C, 44·1; H, 3·4; Cl, 14·4; N, 5·7. Calc. for $C_9H_8ClNO_5$: C, 44·1; H, 3·3; Cl, 14·5; N, 5·7%). When recrystallized from ethanol the compound melted partly at 66°, underwent a resolidification and remelted at 82–84°.

5-Chloro-3,4-dimethoxy-2-nitrobenzyl Benzoate

Sodium borohydride (0·1 g) was added with stirring to the aldehyde (8) (0·5 g) in methanol (2 ml) at −10°, and the solution was allowed to warm to room temperature before being poured into ice water (5 ml). The oily precipitate was extracted with ether, the ether solution dried and the solvent removed to give the corresponding benzyl alcohol (9) (0·47 g, 96%) which was characterized by conversion into the *benzoate* with benzoyl chloride and aqueous sodium hydroxide. Recrystallized from ethanol it had m.p. 86–87° (Found: C, 54·6; H, 4·1; Cl, 10·1; N, 4·0. $C_{16}H_{14}ClNO_6$ requires C, 54·6; H, 4·0; Cl, 10·1; N, 4·0%).

2,8,14-Trichloro-6-ethoxy-3,4,9,10,15,16-hexamethoxy-5,6-dihydrotribenzo[b,f,j][*1,5,9*]*triazacyclododecine*

The nitro aldehyde (8) (1·27 g) in ethanol (100 ml) and hydrochloric acid (2 ml, conc.) was shaken under hydrogen with palladium on charcoal (0·5 g, 5%) for 20 h. Concentration of the solvent to 10 ml caused the precipitation of the *trimer ethanol adduct* (0·15 g, 15%). Recrystallized from carbon tetrachloride/light petroleum then ethanol it gave white needles, m.p. 226–228° [Found: C, 54·4; H, 4·6; Cl, 16·3; N, 6·4; *m/2e* 318·5. $C_{29}H_{30}Cl_3N_3O_7$ requires C, 54·5; H, 4·7; Cl, 16·7; N, 6·6%; mol. wt, 637 (^{35}Cl)]. N.m.r. δ 7·04, 7·01, 6·89 (s, s, s, ArH), 5·46 (2H, s, H12,18), 4·02–3·93 (22H, m, 6Me, CH_2CH_3, CH_2, H5,6), 1·46 (3H, t, CH_2CH_3).

(E)-*5-Chloro-3,4-dimethoxy-2,β-dinitrostyrene (11)*

5-Chloro-3,4-dimethoxy-2-nitrobenzaldehyde (8) (12·3 g) was suspended in ethanol (150 ml) and nitromethane (4·2 g) and cooled to −15°. Potassium hydroxide (12 g) in water (16 ml) was diluted with ethanol (150 ml) and added dropwise over 2 h with stirring in an ice bath. The slurry was then acidified with hydrochloric acid (30 ml, concentrated), diluted with water (200 ml) and extracted with chloroform (3 × 50 ml). The extracts were dried and the solvent was removed to give an oil which was heated under reflux with acetic anhydride (50 ml) and sodium acetate (5 g) for 0·5 h. Pouring into water gave the crystalline *styrene* (13·6 g, 94%). Recrystallized from ethanol it had m.p. 148–149° (Found: C, 42·0; H, 3·0; Cl, 12·7; N, 9·6. $C_{10}H_9ClN_2O_6$ requires C, 41·6; H, 3·1; Cl, 12·3; N, 9·7%). N.m.r. δ ([D_5]pyridine) 8·12 (1H, d, *J* 13·3 Hz, α-H), 7·76 (1H, d, β-H), 7·70 (1H, s, H6), 3·72 (3H, s, OMe), 3·69 (3H, s, OMe).

1-(5-Chloro-3,4-dimethoxy-2-nitrophenyl)-2-nitroethanol (10)

A sample of the oil from the above reaction was heated under reflux in toluene until all the water formed had been removed. On cooling the solution, the *nitroethanol* crystallized. Recrystallized (×2) from benzene it had m.p. 104–105° (Found: C, 39·4; H, 3·4; Cl, 11·8; N, 9·3. $C_{10}H_{11}ClN_2O_7$

requires C, 39·2; H, 3·6; Cl, 11·6; N, 9·1%). N.m.r. δ ($CDCl_3$) 7·46 (1H, s, H6′), 5·74 (1H, m, H1), 4·61 (1H, d, $J_{1,2}$ 5 Hz, H2$_a$), 4·59 (1H, d, $J_{1,2'}$ 7 Hz, H2$_b$), 3·99 (3H, s, OMe), 3·94 (3H, s, OMe), 3·32 (1H, d, $J_{1,OH}$ 4 Hz, OH).

*5-Chloro-6,7-dimethoxy-1*H*-indole* (*19*)

The styrene (11) (5·77 g) was added to aqueous acetic acid (50 ml, 1 : 4) at 50°; iron powder (2 g, SDH Chemicals, hydrogen reduced) was then added and the mixture was warmed until boiling. When spontaneous boiling ceased, further iron powder (12 g) and acetic acid (30 ml) were added slowly so that the mixture remained hot and fluid, and, after the additions, stirring was continued for 0·5 h. Water (200 ml) was added and the mixture was extracted with chloroform (3 × 20 ml). The extracts were washed with aqueous sodium hydrogen carbonate, dried over sodium sulfate and the solvent was removed under vacuum to leave an oil which was extracted with hot light petroleum (3 × 10 ml). Cooling of the extracts gave the white *indole* (19) (1·47 g, 35%). Recrystallized from light petroleum it had m.p. 73–75° (Found: C, 57·0; H, 4·7; Cl, 16·9; N, 6·5. $C_{10}H_{10}ClNO_2$ requires C, 56·9; H, 4·8; Cl, 16·8; N, 6·6%). N.m.r. δ (CCl_4) 8·45 (1H, m, NH), 7·23 (1H, s, H4), 6·94 (1H, q, $J_{1,2}$ 2 Hz, $J_{2,3}$ 3 Hz, H2), 6·26 (1H, q, $J_{1,3}$ 2 Hz, H3), 3·94 (3H, s, OMe), 3·84 (3H, s, OMe).

*5-Chloro-6,7-dimethoxy-*N*-methyl-1*H*-indole* (*20*)

The chlorodimethoxyindole (19) (1·69 g) was methylated according to the method of Potts and Saxton[15] to give a brown oil which was resolved by preparative t.l.c. to give the *N*-methylindole (1·46 g, 81%). Recrystallized from light petroleum it had m.p. 50–50·5° (lit.[3] 44–45°) (Found: C, 58·5; H, 5·3; Cl, 16·0; N, 6·0. Calc. for $C_{11}H_{12}ClNO_2$: C, 58·5; H, 5·4; Cl, 15·7; N, 6·2%). N.m.r. δ ($CDCl_3$) 7·38 (1H, s, H4), 6·92 (1H, d, $J_{2,3}$ 3 Hz, H2), 6·35 (1H, d, H3), 4·01, 3·94, 3·92 (9H, 3s, 3Me).

*5-Chloro-6,7-dimethoxy-*N*-methyl-1*H*-indol-3-yloxoacetic Acid* (*22*)

The *N*-methylindole (20) (0·90 g) was dissolved in anhydrous ether (20 ml), cooled to −20°, and oxalyl chloride (0·5 ml) was added dropwise with stirring over 1 min during which a precipitate of the indol-3-yloxoacetyl chloride formed. After 5 min, sodium hydroxide (0·40 g) in water (10 ml) was added and the stirring was continued for 10 min during which the corresponding *acid* (1·02 g, 86%) precipitated. Recrystallized (× 2) from benzene it had m.p. 185–187° (Found: C, 52·8; H, 4·0; Cl, 12·3; N, 4·8. $C_{13}H_{12}ClNO_5$ requires C, 52·5; H, 4·1; Cl, 11·9; N, 4·7%).

*Ethyl 5-Chloro-6,7-dimethoxy-*N*-methyl-1*H*-indol-3-yloxoacetate*

The indole (0·135 g) was converted into the insoluble substituted oxoacetyl chloride mentioned above to which was added dry ethanol (0·5 ml). After 1 min the solution was poured into saturated aqueous sodium hydrogen carbonate and the mixture was extracted with ether (2 × 10 ml). Drying of the ether solution and removal of the solvent gave the solid ester (0·15 g, 77%). Recrystallized from light petroleum it had m.p. 117–118°C (Found: C, 55·1; H, 4·9; Cl, 11·2; N, 4·0. $C_{15}H_{16}ClNO_5$ requires C, 55·3; H, 5·0; Cl, 10·9; N, 4·3%).

*6,7-Dimethoxy-1*H*-indole* (*17*)

(E)-3,4-Dimethoxy-2,β-dinitrostyrene (2·54 g) was reduced with iron powder and acetic acid in the same manner as was the 5-chloro derivative to give the crude indole (1·31 g, 74%) after preparative t.l.c. Recrystallized from methanol it had m.p. 101–102° (lit.[22] 102–103°). N.m.r. δ ($CDCl_3$) 8·50 (1H, m, NH), 7·38 (1H, d, J 8·5 Hz, ArH), 7·11 (1H, q, $J_{1,2}$ 2·4 Hz, $J_{2,3}$ 3 Hz, H2), 6·92 (1H, d, ArH), 6·53 (1H, q, $J_{1,3}$ 2·4 Hz, H3), 4·03, 3·94 (6H, 2s, 2OMe).

[22] Benington, F., Morin, R. D., and Clark, L. C., *J. Org. Chem.*, 1959, **24**, 917.

6,7-Dimethoxy-N-*methyl-1*H-*indole* (*18*)

The dimethoxyindole (1·06 g) was methylated by the method of Potts and Saxton[15] to give the N-*methylindole* which was obtained after preparative t.l.c. as a colourless oil (0·96 g, 84%) (Found: C, 68·8; H, 6·9; N, 7·0. $C_{11}H_{13}NO_2$ requires C, 69·1; H, 6·9; N, 7·3%). N.m.r. δ ($CDCl_3$) 7·03 (1H, d, *J* 8 Hz, ArH), 6·56 (1H, d, ArH), 6·51 (1H, d, *J* 3 Hz, H2), 6·17 (1H, d, H3), 3·72, 3·65, 3·65 (9H, 2s, 3Me).

6,7-Dimethoxy-N-*methyl-1*H-*indol-3-yloxoacetic Acid* (*21*)

The *N*-methylindole (18) (1·91 g) was treated with oxalyl chloride (1·0 ml) in similar manner to its 5-chloro derivative and gave the *oxo acid* (21) (2·29 g, 87%). Recrystallized from benzene it had m.p. 145–150° (Found: C, 59·6; H, 4·9; N, 5·2. $C_{13}H_{13}NO_5$ requires C, 59·4; H, 5·0; N, 5·3%). N.m.r. δ ($CDCl_3/CD_3SOCD_3$) 8·13 (1H, s, H2), 7·83 (1H, d, *J* 8·5 Hz, ArH), 6·89 (1H, d, H5), 5·84 (1H, s, COOH), 3·98, 3·87, 3·85 (9H, 3s, 3Me).

N-*Methyl-1*H-*indol-3-yloxoacetic Acid* (*16*)

N-Methylindole (13) (1·31 g) was treated with oxalyl chloride (1·0 ml) in the same way as were the substituted derivatives to give the acid (1·58 g, 78%). Recrystallized from chloroform/light petroleum it had m.p. 155–158° (lit.[23] 160–162°). N.m.r. δ ($CDCl_3/CD_3SOCD_3$) 8·50 (1H, s, H2), 8·42 (1H, m, H4), 8·35 (1H, s, COOH), 7·50–7·20 (3H, m, H5–7), 3·86 (3H, s, Me).

Dimethyl(*1'-methyl-1'*H-*indol-3'-ylmethylene*)*ammonium Chloride* (*23*)

Phosphoryl chloride (5 ml) was added to *N,N*-dimethylformamide (25 ml) at ice-bath temperature and the solution was stirred for 10 min. *N*-Methylindole (5·25 g) was added dropwise and the slurry formed was allowed to stand for 0·5 h; carbon tetrachloride (50 ml) was then added and the mixture was stirred for 5 min. The white precipitate was washed with carbon tetrachloride and dried under vacuum to give the *chloride* (23) (8·78 g, 98%). Recrystallization was effected by dissolving in a minimum volume of cold water and pouring into excess of acetone. The compound decomposed above 200° (Found: C, 64·6; H, 6·8; N, 12·5. $C_{12}H_{15}ClN_2$ requires C, 64·7; H, 6·8; N, 12·6%). N.m.r. δ (D_2O) 8·06, 7·77 (2H, 2s, H2', CH=N), 7·7 (4H, m, H4',5',6',7'), 3·68 (6H, s, $^+$NMe), 3·24 (3H, s, NMe).

Dimethyl(*1'-methyl-1'*H-*indol-3'-ylmethylene*)*ammonium Iodide* (*24*)

To the chloride (23) (0·67 g) in hot methanol (20 ml), sodium iodide (1 g) in methanol (20 ml) was added. The precipitated *iodide* (24) (0·88 g, 93%) was filtered off after cooling and recrystallized from methanol. It decomposed at *c.* 300° (Found: C, 46·0; H, 4·7; I, 40·8; N, 9·2. $C_{12}H_{15}IN_2$ requires C, 45·8; H, 4·8; I, 40·4; N, 8·9%).

N-*Methyl-1*H-*indole-3-carbaldehyde* (*15*)

This was prepared by formylation of *N*-methylindole or by methylation of indole-3-carbaldehyde with methyl iodide and potassium carbonate with acetone as solvent. *N*-Methylindole (13) was prepared by the method of Potts and Saxton[15] and indole-3-carbaldehyde following the method of James and Snyder.[24]

Dimethyl (*1'-Methyl-1'*H-*indol-3'-ylmethylene*)*malonate* (*25*)

The salt (23) (1·1 g) and dimethyl malonate (0·73 g) were heated under reflux for 1 h in dry methanol (20 ml) containing sodium methoxide (3 mg). Removal of the solvent gave a solid residue which, recrystallized from methanol, was the *malonate* (25) (0·7 g, 52%), m.p. 99–100° (Found: C, 65·7; H, 5·7; N, 5·0. $C_{15}H_{15}NO_4$ requires C, 65·9; H, 5·5; N, 5·1%).

[23] Chamberlain, K., and Wain, R. L., *Ann. Appl. Biol.*, 1973, **75**, 409.
[24] James, P. N., and Snyder, H. R., *Org. Synth.*, 1963, Coll. Vol. IV, 539.

*Diethyl (1'-Methyl-1'*H*-indol-3'-ylmethylene)malonate (26)*

Reaction of the salt (23) at room temperature with diethyl malonate in ethanol containing sodium ethoxide likewise gave the *diethyl ester* (26) in 42% yield. Recrystallized from methanol it had m.p. 75–77° (Found: C, 67·9; H, 6·4; N, 4·5. $C_{17}H_{19}NO_4$ requires C, 67·8; H, 6·4; N, 4·7%).

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