Mass Spectrometric and Nuclear Magnetic Resonance Confirmation of a 3,3-Spirocyclic Indole Derivative Formed from Melatonin and Related Acyl Tryptamines†

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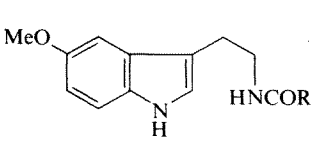
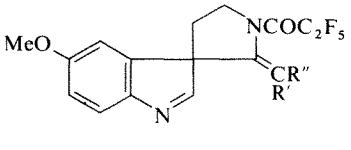
When melatonin is reacted with pentafluoropropionic anhydride for selected ion monitoring or electron capture gas chromatographic analysis a volatile product results. Examination of the product by combined gas chromatography mass spectrometry has established that the molecular weight of 360 corresponds to the addition of one molecule of pentafluoropropionic acid followed by dehydration. Further spectroscopic and chemical examination using mass spectrometry and nuclear magnetic resonance with isotopic labelling has established that the product is a 3,3-spirocyclic indole derivative. Several analogous compounds were also examined and their mass spectra studied.

INTRODUCTION

Melatonin (**1**), produced in the pineal body of vertebrates, occurs in body fluids at such low concentrations that its determination demands highly sensitive and specific procedures. Cattabeni, Koslow and Costa[1] used gas chromatography mass spectrometry with selected ion monitoring of a pentafluoropropionyl (PFP) derivative prepared by heating melatonin with excess pentafluoropropionic anhydride (PFPA) and attributed the structure **2** to the product, because of the known tendency for such compounds to be formed under dehydrative conditions.[2] However, when we prepared a PFP derivative of 6-methoxyharmalan (**3**) by reaction with 1-pentafluoropropionylimidazole (PFPI) rather than with PFPA (which generally contains some free acid), the mass spectrum and chromatographic properties on t.l.c. and g.l.c. of this product (**2**) were quite different from those of the product from the reaction of melatonin with PFPA (**4**) (Table 1).

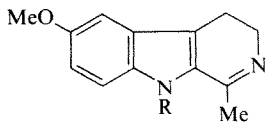




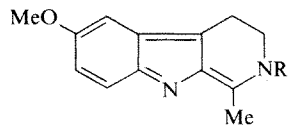
1: R = Me
7: R = CD_3
8: R = Et
9: R = Pr
10: R = Pr^i
11: R = Bu^t
17: R = C_2F_5

4: R′ = R″ = H
12: R′ = R″ = D
13a: R′ = H, R″ = Me
13b: R′ = Me, R″ = H
14a: R′ = H, R″ = Et
14b: R′ = Et, R″ = H
15: R′ = R″ = Me

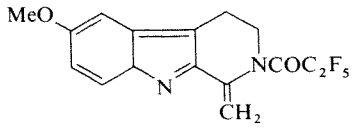
† Abbreviations: PFP = pentafluoropropionyl; PFPA = pentafluoropropionic anhydride; PFPI = 1-pentafluoropropionylimidazole.



2: R = COC_2F_5
3: R = H



5: R = COC_2F_5
6: R = H



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EXPERIMENTAL

G.l.c. analyses were performed on a Hewlett-Packard 5710A chromatograph with an electron capture detector and a 400 × 0.25 cm glass column of 3% silicone

Table 1. Gas chromatographic data: 400 × 0.25 cm column of 3% OV-225 on 100/120 mesh Gas Chrom Q at 215 °C. G.l.c. retention data relative to the PFP derivative of 1,10-diaminodecane which had t_R 14.9 min

Compound	Rel. t_R
2	3.58
TFA derivative of **1**	1.46
4	1.09
HFB derivative of **1**	0.98
13	1.11 1.18
14	1.07 sh 1.19
15	1.28
17	1.46

Table 2. Mass spectral data for acylated 5-methoxytryptamines and derived PFP-cyclization products (*m/e* and % of base peak) (unless indicated, samples were introduced via the g.c. column)

N-acetyl-5-methoxytryptamine (melatonin, **1**) (direct inlet)	233 (3), 232 (19; [M]$^{+}$), 174 (11), 173 (84), 161 (11), 160 (100), 158 (11), 145 (23), 117 (23), 43 (19), 30 (17).
N-($^{2}H_3$ acetyl)-5-methoxytryptamine (**7**) (direct inlet)	236 (3), 235 (17; [M]$^{+}$), 174 (12), 173 (86), 161 (12), 160 (100), 158 (10), 145 (19), 117 (17), 46 (12), 31 (10).
3,3-Spirocyclic TFA-derivative of melatonin	312 (3), 311 (34), 310 (100; [M]$^{+}$), 309 (20), 213 (70), 198 (24), 186 (46), 184 (18), 170 (26), 169 (15), 115 (14).
3,3-Spirocyclic PFP-derivative (**4**) made from melatonin (**1**), Fig. 3	362 (3), 361 (26), 360 (100; [M]$^{+}$), 359 (11), 241 (12), 213 (57), 198 (19), 186 (33), 184 (18), 170 (18), 169 (12).
3,3-Spirocyclic PFP-derivative (**12**) from deuterated melatonin (**7**) Fig. 4	364 (2), 363 (22), 362 (100, [M]$^{+}$), 361 (13), 215 (45), 200 (14), 188 (26), 186 (15), 173 (16), 172 (19), 171 (11).
3,3-Spirocyclic HFB-derivative of melatonin	412 (3), 411 (19), 410 (100; [M]$^{+}$), 241 (9), 213 (47), 198 (16), 186 (25), 184 (15), 170 (13), 169 (12), 101 (10).
PFP-derivative (**2** or **5**) from 6-methoxyharmalan (**3** or **6**), Fig. 5	362 (2), 361 (9), 360 (38; [M]$^{+}$), 242 (15), 241 (100), 226 (5), 198 (6), 184 (6), 169 (6), 120 (11).
3,3-Spirocyclic PFP-derivative (**13**) made from *N*-propionyl-5-methoxytryptamine (**8**)	375 (19), 374 (100; [M]$^{+}$), 373 (49), 359 (27), 347 (18), 346 (45), 255 (34), 227 (21), 199 (31), 174 (44).
3,3-Spirocyclic PFP-derivative (**14**) made from *N*-*n*-butyryl-5-methoxytryptamine (**10**)	390 (12), 389 (16), 388 (76; [M]$^{+}$), 374 (20), 373 (100), 360 (21), 359 (85), 269 (22), 212 (15), 198 (14), 174 (39).
3,3-Spirocyclic PFP-derivative (**15**) made from *N*-isobutyryl-5-methoxytryptamine (**11**)	389 (12), 388 (57; [M]$^{+}$), 387 (11), 373 (24), 319 (33), 306 (15), 270 (18), 269 (100), 254 (22), 226 (14), 225 (13), 198 (13).

OV-225 on 100/120 mesh Gas Chrom Q at 215 °C. Mass spectra were determined on an LKB 9000S combined g.c.m.s. instrument at 70 eV. N.m.r. spectra were recorded at 100 MHz on a Varian HA-100 in $CDCl_3$. U.v. spectra were recorded on a Unicam SP 800. T.l.c. analyses were carried out on Merck glass plates precoated with silica gel 60 F_{254}.

N^{α}-[$^{2}H_3$]Acetyl-5-methoxytryptamine (7)

5-Methoxytryptamine (13.8 mg) was dissolved in 0.5 ml chloroform and trideuteroacetyl chloride (2.8 μl, $\frac{1}{2}$ equiv.) was added. An immediately formed precipitate of 5-methoxytryptamine hydrochlordie was extracted twice with 1 ml 0.02 M HCl and evaporation of the chloroform to dryness gave trideuterated melatonin in almost 50% yield based on the starting material. T.l.c. showed the product to be pure and indistinguishable from authentic melatonin, and its mass spectrum, obtained by direct insertion, was similar to that of melatonin except for a molecular ion at *m/e* 235 and a deuterioacetyl fragment of *m/e* 46 (Table 2).

Preparation of other Nα-acylated-5-methoxytryptamines

These were made as above from 5-methoxytryptamine in chloroform with a half-equivalent of the appropriate acid chloride: propionyl chloride gave **8**; iso-butyryl chloride gave **9**; *n*-butyryl gave **10**; and pivaloyl chloride gave **11**. These compounds all showed a single spot by t.l.c. and had the correct mass spectra obtained via direct insertion.

Cyclization of melatonin (1) and of trideuteriomelatonin (7) with PFPA

Melatonin (72 mg) was dissolved in 2% PFPA in dry A.R. benzene (50 ml) at 0 °C, and after 10 min the solution was evaporated to dryness on a rotary evaporator. The yellow residue was dissolved in chloroform (1 ml) and layered onto the top of a 20× 0.9 cm column of silica gel (BDH, 60/120 mesh for chromatography) packed from a slurry with chloroform. Elution was with chloroform, and after rejection of the first 10 ml, the next 40 ml emerging ahead of the orange zone were collected and evaporated to dryness to give the spiro derivative (**4**, 108 mg, 97%) as a pale yellow oil which crystallized on standing. The dideuterio analogue (**12**) was prepared in the same way. Cyclization with trifluoroacetic anhydride or heptafluorobutyric anhydride gave analogous products under these conditions. All these substances were found to be pure by t.l.c. and g.l.c. and by g.c.m.s. Mass spectra of the PFP derivatives are shown in Figs. 3 and 4, and Figs. 1 and 2 show their n.m.r. spectra. Mass spectra of the other derivatives are contained in Table 2. The u.v. spectrum has λ_{max} (CH_3OH) 224 (ε 5 370), 267 (8 910) and 300 sh (approx. 5 800) nm.

Cyclization of other acyl 5-methoxytryptamines

N-Propionyl-5-methoxytryptamine (**8**, 500 μg) was treated with PFPA (100 μl) for 30 min at 65 °C and evaporated to dryness in a stream of N_2 to give the

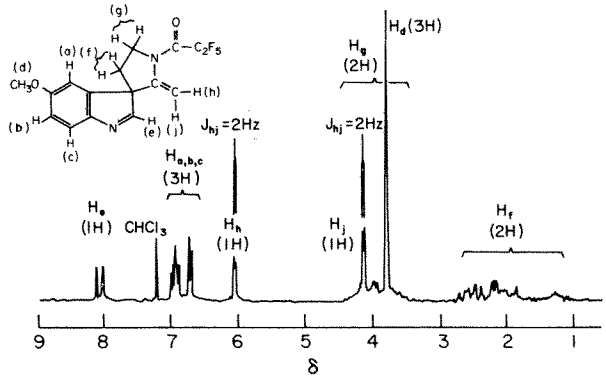


Figure 1. ^{1}H n.m.r. spectrum of melatonin derivative (**4**).

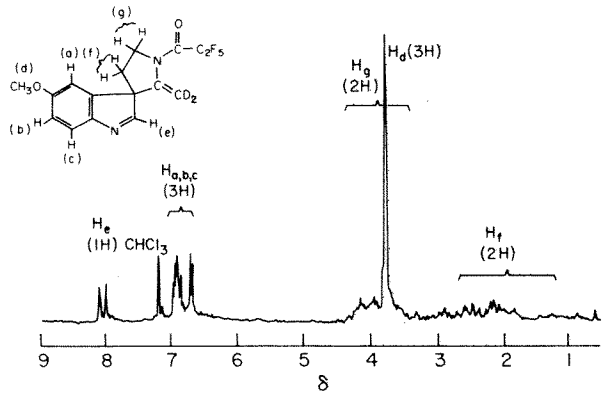


Figure 2. ^{1}H n.m.r. spectrum of deuteriated (**12**) analogue of compound **4**.

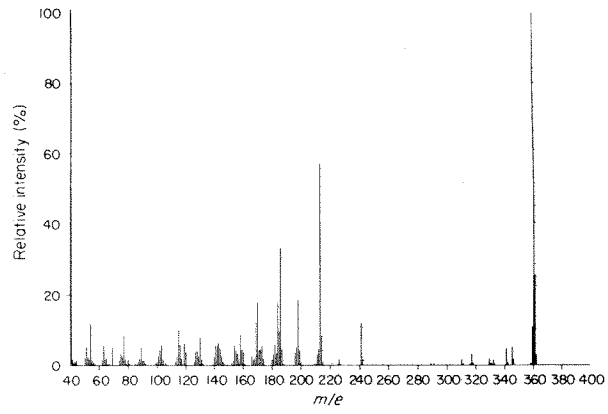


Figure 3. Mass spectrum of compound **4** derived from melatonin (**1**).

3-spiro derivative (**13**) as yellow oil pure by t.l.c. and g.l.c. *N*-Butyryl-5-methoxytryptamine (**9**) gave the 3-spiro derivative (**14**) after 1½ h at 65 °C. The isobutyryl-5-methoxytryptamine (**10**) did not cyclize under these conditions; minimal amounts of the cyclized product (**15**) were detected by g.c.m.s. after heating at 65 °C overnight, but the predominant reaction was displacement of the isobutyryl group to give *N*-PFP-5-methoxytryptamine (**17**), which was also the major product of the attempted cyclization of *N*-pivaloyl-5-methoxytryptamine (**11**) at 100 °C for 24 h.

PFP derivative of 6-methoxyharmalan

6-Methoxyharmalan (**3**, 21 mg) was dissolved in PFPI (100 μl) and left overnight at 20 °C, diluted with chloroform (1 ml) and extracted with 2 × 1 ml 0.02 M HCl and 1 ml water. The chloroform solution was evaporated to dryness and the orange oil crystallized overnight at 5 °C. T.l.c. and g.l.c. showed the product to contain some impurities, which were removed by chromatography on silica gel, as previously described, with collection of the main coloured fraction and evaporation to dryness. The u.v. spectrum has λ_{max} 220 (ε 12 240), 285 (22 900) and 325 sh (approx. 9 360) nm. The mass spectrum of **2** is shown in Fig. 5.

RESULTS AND DISCUSSION

The mass spectrum of the product from melatonin and PFPA (Fig. 3) was compared with that of the product of 6-methoxyharmalan and PFPI (Fig. 5). They were found to be similar, but with marked differences in the relative intensities of the molecular, $[M-C_2F_5]^+$ and $[M-C_2F_5CO]^+$ ions. The trifluoroacetyl and heptafluorobutyryl derivatives of melatonin were prepared and examined by g.c.m.s.: mass shifts of ±50 a.m.u. (±CF_2) from the *m/e* 360 molecular ion of the PFP derivative established the presence of a single C_2F_5 function. Mass measurement of the molecular ions of the isomers at *m/e* 360 confirmed a molecular formula of $C_{16}H_{13}N_2O_2F_5$ for both derivatives. Additional spectroscopic and chemical evidence prompts us to propose the unexpected structure **4**, formed via cyclization to the 3-position of the indole nucleus, for the product of the reaction between melatonin and PFPA. Neither derivative gave an Ehrlich colour-reaction for indoles. The ability to distinguish between **2** and **4** is important, because it has been suggested that compounds such as **3** might be produced by the normal metabolism of melatonin,[3] and any method of derivatization in which an identical product such as **2** is formed from both **1** and **3** is undesirable as it would make interpretation of the results difficult. The spiro structure of **4** is of interest as a further example of the electrophilic reactivity of the 3-position of the indole nucleus even when already substituted.[4–9]

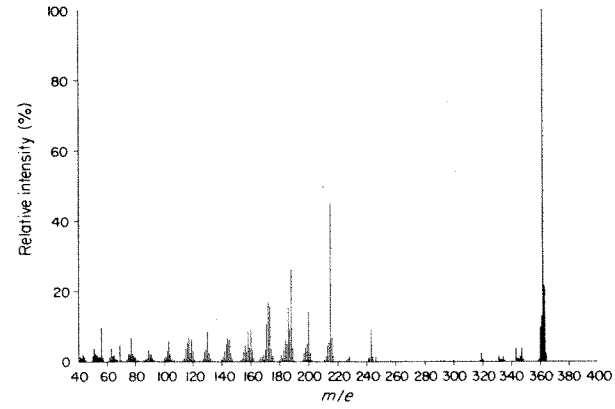


Figure 4. Mass spectrum of compound **12** derived from deuteriomelatonin (**7**).

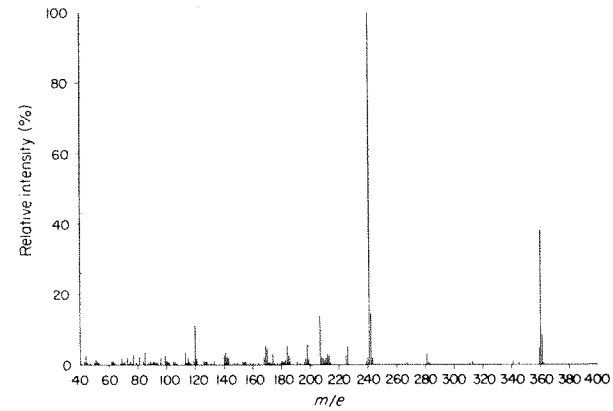
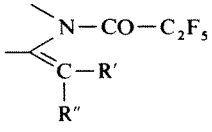


Figure 5. Mass spectrum of the product (**2**) of the reaction of 6-methoxyharmalan (**3**) with pentafluoropropionyl imidazole.

We have prepared **4** in almost quantitative yield, for determination by g.l.c. with electron capture detection down to $>10^{-10}$ g. This derivative of melatonin is also satisfactory for selected ion monitoring analysis because a high proportion of the total ion current is carried by the molecular ion. Melatonin was dissolved in a dilute solution of PFPA in benzene at 0 °C and reacted almost instantaneously: prolonged reaction or heating only leads to the formation of polymeric and coloured materials. The product may contain traces (<1%) of an isomer with chromatographic properties identical with those of the compound produced from **3** and PFPI: this compound may be separated by liquid chromatography on silica gel.

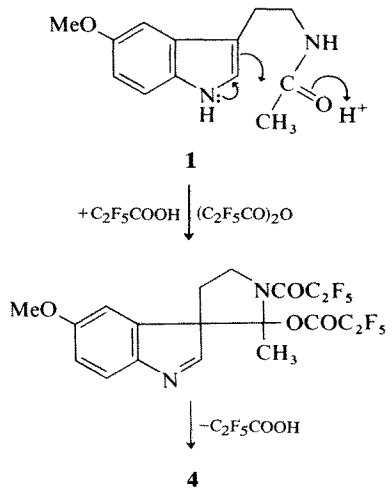
Ultraviolet spectra of the two isomers after purification in this way showed that the melatonin derivative no longer had an intact indole chromophore: λ_{max} (CH_3OH)224 (ε 5 370), 267 (8 910) and 300 sh nm. The indole chromophore of melatonin has λ_{max} (CH_3OH) 278, 298 and 310 nm. The derivative formed by the reaction of **3** with PFPI had λ_{max} (CH_3OH) 220 (ε 12 240), 285 (22 900) and 325 sh nm; the starting material **3** had λ_{max} (CH_3OH) 325 (ε 12 880) and 370 sh nm. In our experience PFPI does not promote rearrangements and dehydration to the same extent as PFPA, because it does not give acidic by-products and forms derivatives by an exchange process. It seems probable from the u.v. evidence that the product from reacting **3** with PFPI is a mixture of structures **2** and **5**. An equilibrium between **3** and **6** may also exist, and since the pyridine nitrogen is more basic than the indole nitrogen, the formation of structure **5** would probably be favoured.

Deuterium labelled melatonin (**7**) was prepared from 5-methoxytryptamine and [$^2H_3$]acetyl chloride of >99% isotopic purity. The reaction of **7** with PFPA gave a derivative which contained only two of the deuterium atoms of the starting material. Mass spectrometry showed this derivative to consist of <1% monodeuteriated, >98% dideuteriated and <1% trideuteriated material (Fig. 4). *N*–Propionyl-5-methoxytryptamine (**8**) gave a product (**13**) with a molecular ion at *m/e* 374 and abundant $[M-1]^+$, $[M-15]^+$ and $[M-28]^+$ ions (see Table 2); *N*-*n*-butyryl-5-methoxytryptamine (**9**) gave a product (**14**) with a molecular ion at *m/e* 388 and abundant $[M-15]^+$, $[M-28]^+$ and $[M-29]^+$ ions. This indicated that in addition to loss of C_2H_4, probably due to a hydrogen rearrangement process, some form of electron impact induced β-cleavage was occurring, giving rise to losses of $H^\cdot$ and $CH_3^\cdot$, or of $CH_3^\cdot$ and $C_2H_5^\cdot$; this, together with the deuterium labelling results, suggested that the acyl functions are transformed into an '*exo*' double bond:



N-Isobutyryl-5-methoxytryptamine (**10**) could only be cyclized to the 3-spiro derivative (**15**) in very low yield and under much more vigorous conditions. The way in which the difficulty of preparation increases with increasing substitution at the α-carbon of the acyl group is further evidence for its transformation into an unsaturated grouping. *N*-Pivaloyl-5-methoxytryptamine (**11**), lacking an α-hydrogen to eliminate, did not cyclize even under the most vigorous conditions: displacement of the pivaloyl by a PFP group was the only reaction.

Jackson and co-workers have investigated electrophilic substitution in indoles extensively,[4–12] and their results point to the distinct possibility of cyclization to the already substituted 3-position of the indole nucleus with the formation of a 3-spiro compound. Jackson and Smith[4] concluded that direct electrophilic substitution at the 2-position of the indole nucleus is energetically unfavourable compared with attack at the 3-position, because the intermediate would have a structure in which the π-electron system of the benzene ring is disturbed. Substitution at the 3-position does not disturb the benzene π-electrons, and the indole is behaving in an analogous fashion to an enamine. We considered the possibility of cyclization to the 2-position, but not only is this argument against it, but the product (**16**) from such a reaction would be expected to resemble **2** or **5** in spectroscopic and chromatographic properties much more closely than was actually found. Precedents exist for an intramolecular cyclization of this type suggested in the scheme; 4-(3-indolyl)butanol undergoes BF_3-catalysed cyclization via a 3,3-spirocyclopentanoindolenine[5] and indoalkyl tosylates undergo solvolysis with the anchimeric assistance of the 3-position.[6] The suggested reaction scheme for formation **4** is shown in Scheme 1.



Scheme 1. Proposed mechanism for the formation of compound **4** from melatonin

Samples of both the melatonin and deuteriomelatonin PFP-derivatives were examined by ^1H n.m.r. in deuteriochloroform at 100 MHz (Figs 1 and 2). The signals at δ6.1 (1H, *J* 2 Hz) and δ4.18 (1H, *J* 2 Hz) were absent in the deuteriated sample: these resonances appear to be spin-coupled. Considering the coupling constant, chemical shifts and starting material, these must correspond to a vinylic methylene group with the two protons in substantially different environments. This is in agreement with earlier deductions concerning the *exo* double bond. Comparison with the chemical shift observed by Jackson and Smith[4] for monomeric 3-spiro indolenines, showed that the doublet at δ8.1

(1H, *J* 9 Hz) is probably due to the proton at position 2 of the indole nucleus. Apart from the methoxy signal at δ3.84 (3H, s) and aromatic signals at δ7.03–δ6.70 (3H, m), the integral indicated multiplet signals at δ4.5–δ3.6 (2H) and δ2.7–δ1.9 (2H) due to the aliphatic 5-membered ring protons, those at δ4.5–δ3.6 are shifted downfield by the effect of the fluoroamido function.

An examination of a three-dimensional model of structure **4** indicated that the two vinylic protons are in very different environments; the proton *trans* to the amido function would probably suffer a considerable shift due to the influence of the aromatic ring current, and the proton *cis* to the amido function would be shifted by the influence of the carbonyl and fluorine-containing group. The derivative **13** formed from *N*-propionyl-5-methoxytryptamine (**8**) was separated into equal quantities of two isomeric components by g.c.m.s., and the mass spectra were indistinguishable. This can be explained by the formation of the two geometrical isomers (**13a**) and (**13b**).

The ease of formation of **4** suggests a stable structure: it was in fact found to be resistant to attack by dilute alkalis and acids, and these properties, together with its electron-capturing ability, make it suitable for the sensitive quantitative determination of melatonin. This general approach can also be used for the determination of compounds that may be converted into acetylated typtamines and tryptophan derivatives. We are also exploring its potential in the specific determination of other tryptophan and tryptamine derivatives.

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REFERENCES

1. F. Cattabeni, S. H. Koslow and E. Costa, *Science* **178**, 166 (1972).
2. E. Spath and E. Lederer, *Chem. Ber.* **63**, 2102 (1930).
3. S. Kveder and W. M. McIsaac, *J. Biol. Chem.* **236**, 3214 (1961).
4. A. H. Jackson and A. E. Smith, *Tetrahedron* **24**, 403 (1968).
5. A. H. Jackson, B. Naidoo and P. Smith, *Tetrahedron* **24**, 6119 (1968).
6. K. M. Biswas and A. H. Jackson, *Tetrahedron* **25**, 227 (1969).
7. A. H. Jackson and B. Naidoo, *Tetrahedron* **25**, 4843 (1969).
8. A. H. Jackson and B. Naidoo, *J. Chem. Soc. Perkin Trans. 2* 548 (1973).
9. R. Iyer, A. H. Jackson, P. R. Shannon and B. Naidoo, *J. Chem. Soc. Perkin Trans. 2* 872 (1973).
10. R. Iyer, A. H. Jackson and P. R. Shannon, *J. Chem. Soc. Perkin Trans. 2* 878 (1973).
11. A. H. Jackson and P. Smith, *Tetrahedron* **21**, 989 (1965).
12. A. H. Jackson and P. Smith, *Tetrahedron* **24**, 2227 (1968).

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