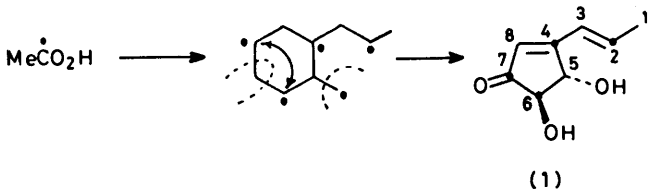
Biosynthesis of Fungal Metabolites. Terrein, a Metabolite of *Aspergillus terreus* Thom [1]

By **Robert A. Hill, Rachel H. Carter,** and **James Staunton,*** University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW

Terrein, a metabolite of *Aspergillus terreus* Thom, is biosynthesised from 3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin by contraction of an aryl ring. The direction of the ring contraction has been investigated using [1,2-$^{13}C_2$]acetate as precursor.

TERREIN (1), a metabolite of *Aspergillus terreus* Thom, was first isolated in 1935 by Raistrick.[2] Early attempts [3] to elucidate the structure were unsuccessful and it was not until 1955 that the problem was finally solved by Grove [4] and by Barton [5] who also established the absolute configuration shown (1). Another nineteen years elapsed before the structure was confirmed by a synthesis of the racemic form of terrein.[6]

The biosynthesis of the metabolite has also presented a long-standing and intriguing problem. Early work by Birch [7] showed that radioactivity was incorporated from [1-^{14}C]acetate in a manner which suggested a polyketide mode of biosynthesis. Thus for the most part the molecule was labelled on alternate carbons as shown in Scheme 1, and, moreover, the distribution of activity



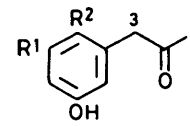
(1)

SCHEME 1

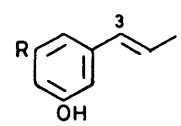
over the labelled positions was uniform. However, there was an unusual break in the alternating pattern at C-6 and C-7, both of which are derived from the carboxy-group of acetate. Parallel incorporation studies with [2-^{14}C]acetate produced complementary results. Further confirmation came from experiments with [2-^{14}C]-malonate which also provided evidence that C-1–C-2 corresponds to the starting unit of the hypothetical ketide chain.[7]

The demonstration that terrein has a direct link between two carbon atoms derived from the carboxy-group of acetate makes this an interesting biosynthetic problem. Another group of metabolites which are known to show this unusual feature are the aflatoxins.[8] Of the several biosynthetic schemes which can be advanced to account for this labelling pattern in terrein the most attractive is one proposed initially by Birch [7] in which the five-membered ring of terrein is formed by contraction of a six-membered ring as shown in Scheme 1.

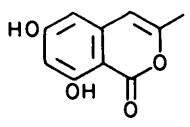
We have tested this hypothesis by synthesising a number of hypothetical aromatic precursors for incorporation studies. Depending on the timing of the various steps (ring contraction, loss of two C_1 units, and generation of the alkene bond in the side chain) a large number of candidates are possible. Compounds (2), (3), (4), (5), (6), and (7) were selected initially for testing;



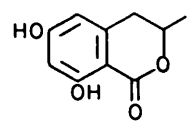
(2) R^1 = OH, R^2 = H
(3) R^1 = H, R^2 = OH



(4) R = OH
(5) R = H



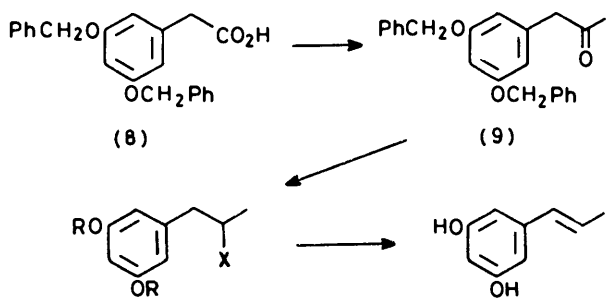
(6)



(7)

the first four correspond to pathways in which one C_1 unit is lost prior to ring contraction, the remaining two make no assumption in this respect.

The synthetic route leading to (2) and (4) is shown in Scheme 2. Treatment with methyl-lithium converted



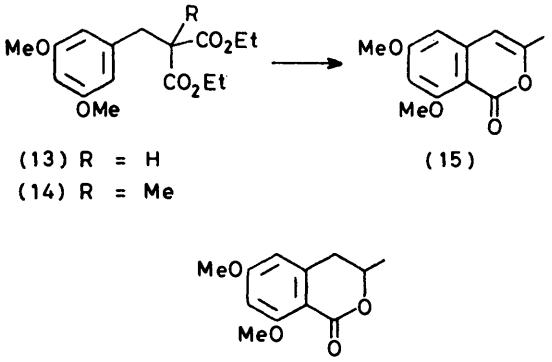
(10) R = CH_2Ph, X = OH
(11) R = CH_2Ph, X = Cl
(12) R = H, X = Cl

SCHEME 2

the phenylacetic acid (8) into the methyl ketone (9) which can be debenzylated to give (2) or converted into the corresponding alkene (4) *via* the sequence (9)→(10)→(11)→(12)→(4) using $NaBH_4$, PCl_5, catalytic hydrogen-

ation, and KOH respectively for successive steps. Compounds (3) and (5) were synthesised by equivalent routes from the corresponding benzyloxyphenylacetic acids. These synthetic routes all proceed in excellent yield. They are also readily adapted for the introduction of a specific radiolabel by using [^{14}C]MeLi (derived from [^{14}C]MeI) in the first step.

The radiochemical synthesis of (6) and (7) is shown in outline in Scheme 3. Methylation of (13) with [^{14}C]MeI



SCHEME 3

gave (14) which was converted to the isocoumarin (15) by a route described earlier.[9] Treatment of (15) with BBr_3 gave (6) in high yield. The corresponding dihydroderivative (7) was also readily prepared from (15) by hydrogenation followed by demethylation.

The results in Table 1 show that radioactivity was

TABLE 1

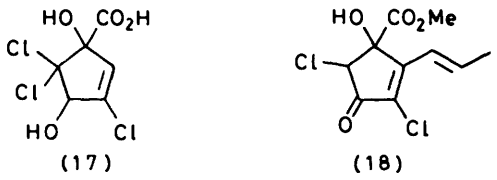
Results of incorporation experiments

Expt. no.	Administered compound	Incorporation (%)	% Label at C-1 + C-2 [a]
1	[*carboxy*-^{14}C]$MeCO_2H$	0.59	24.1
2	[3-$^{14}C_1$]-(2)	0.19	24.4
3	[3-$^{14}C_1$]-(3)	0.75	24.5
4	[3-^{14}C]-(4)	0.01	
5	[3-^{14}C]-(5)	0.01	
6	[*Me*-^{14}C]-(6)	0.04	24.3
7	[*Me*-^{14}C]-(7)	2.27	92.3 [b]

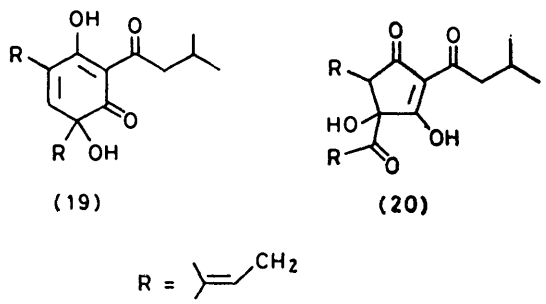
[a] By Kuhn–Roth degradation. Acetic acid isolated as *p*-bromophenacyl acetate. [b] Schmidt degradation of the acetic acid showed that all the activity resided in the methyl group.

incorporated in significant amounts from four of the six potential precursors. In each case an intact incorporation should result in terrein labelled solely at C-1 and so the specificity of incorporation was checked by Kuhn-Roth oxidation to produce acetic acid from C-1 + C-2. The samples of terrein produced in experiments 2, 3, and 6 were each found to carry one quarter of the total activity in the key C_2 unit. This result is consistent with prior degradation of the administered compound to [2-^{14}C]acetate which would then be incorporated in the normal way to produce a labelling pattern complementary to that shown in Scheme 1. In contrast, the terrein derived from (7) yielded acetic acid which carried 92% of the total activity and, as expected, all this activity resided in the terminal methyl group. Thus the evidence is strong that (7) can serve as an intact precursor (that the degree of specificity of incorporation was less than 100% may be attributed to competing degradation of the compound to acetate prior to incorporation). This dihydroisocoumarin has also been reported [10] as a metabolite of *Aspergillus terreus* and so its status as a true biosynthetic intermediate is well supported.

It seems unlikely, however, that this intermediate participates in the key step of the pathway in which the six-membered ring undergoes contraction. Chemical precedents for the process are few but significantly in every case the aryl ring is hydroxylated or chlorinated prior to ring contraction. For example phenol is converted into the Hantzch acid (17) on treatment with



chlorine and base.[11] Recently this type of reaction has been adapted by Strunz [12] to provide the basis of an elegant synthesis of cryptosporiopsin (18). Another reaction which may be more directly relevant to terrein biosynthesis is the conversion of the humulone (19) into the isohumulone (20) on treatment with base.[13]



With these precedents in mind we plan in future to test more highly oxygenated derivatives of (7) as intermediates in terrein biosynthesis. In the meantime we have examined the pattern of incorporation of [1,2-$^{13}C_2$]-acetate, an established technique for probing skeletal change in polyketide biosynthesis,[14] and have obtained results which establish the general nature of the rearrangement process.

The n.m.r. spectral data of terrein which provide the basis for this investigation are given in Tables 2 and 3.

TABLE 2

^{1}H N.m.r. spectrum of terrein (1) in [$^2H_6$]acetone

Position of proton	τ
1	8.11 (3 H, t, *J* 7 Hz)
2	3.18 (1 H, dq, *J* 7 and 16 Hz)
3	3.59 (1 H, d, *J* 16 Hz)
5	5.26br (1 H, d)
6	5.93 (1 H, d, *J* 2 Hz)
8	4.06br (1 H, s)

The signals in the proton spectrum were assigned unambiguously on the basis of chemical shift and coupling pattern (checked where appropriate by homonuclear decoupling experiments) with the exception of those at δ 5.26 and 5.93. One of these was a doublet (*J* 2 Hz), the other an unresolved multiplet which collapsed to a doublet (*J* 2 Hz) on irradiation of the proton attached to C-8. The multiplet was therefore assigned to the proton on C(5) and the doublet to that on C(6).

Six of the eight signals in the ^{13}C n.m.r. spectrum (Table 3) were assigned by correlation with signals in the proton n.m.r. spectrum using standard off-resonance spin-decoupling techniques. [15] The remaining two (at δ 168.3 and 202.6), which did not show strong $^{13}C-^{1}H$ coupling, were assigned as shown on the basis of their chemical shifts.

TABLE 3

^{13}C Chemical shifts and $^{13}C-^{13}C$ coupling

Carbon	δ_C (p.p.m. relative to Me_4Si)	$^{13}C-^{13}C$ coupling in terrein labelled with [1,2-$^{13}C_2$]-acetate (Hz)
1	18.7	42
2	139.5	42
3	125.1	52
4	169.3	52
5	77.2	*a*
6	81.7	*a*
7	202.6	56
8	125.7	56

[a] Singlet.

A preliminary incorporation experiment was carried out using [1-^{13}C]acetate as precursor. The ^{13}C n.m.r. spectrum (Figure 1) of the resultant terrein had four signals of enhanced intensity, *viz.* those assigned to C-2, C-4, C-6, and C-7. This labelling pattern accords with that proposed by Birch on the basis of experiments with [1-^{14}C]acetate. The ^{13}C experiment defines the pattern completely and unambiguously apart from uncertainty in the assignment of the signal at δ 81.7 to C-6 rather than C-5. The origin of C-6 was therefore checked independently by carrying out a parallel incorporation experiment with [1-^{14}C]acetate; degradation of the resultant terrein with periodate [2] gave formic acid from C-6 which, as expected, carried 25% of the total activity. Further evidence that this signal arises from C-6 was provided by the ^{13}C n.m.r. spectrum of the labelled terrein (Figure 1) where it showed a doublet (*J* 42 Hz), of low intensity, symmetrically disposed about the intense singlet. The signal from C-7 showed a complementary coupling pattern. These doublets are attributed to $^{13}C-^{13}C$ coupling between C-6 and C-7; the fact that they can be detected reflects the relatively high degree of isotopic enrichment at the labelled positions (10%) which results in a small but detectable percentage of molecules which are labelled simultaneously at these carbon atoms.

In the ^{13}C n.m.r. spectrum of terrein derived from [1,2-$^{13}C_2$]acetate (Figure 2), six signals showed the characteristic pattern of an intense doublet symmetrically disposed about the singlet arising from ^{13}C at natural abundance. From the coupling constants (Table 3) it was evident that C-1 was coupled to C-2, C-3 to C-4, and C-8 to C-7. The signals from C-5 and C-6 were enhanced in intensity but significantly both were singlets with no intense $^{13}C-^{13}C$ doublet in evidence.

These results show that terrein incorporates three intact C_2 units, the remaining two carbons being derived from separate C_2 units. Our interpretation of them is shown in Scheme 4 (where intact C_2 units are indicated by heavy lines). Thus we propose that the intermediate (7) will be labelled as shown (Scheme 4) and that the destination in terrein of various carbons is as follows: C-5 is converted into C-8, C-6 into C-7, C-8 into C-6, and C-8a into C-5; C-1 and C-7 are both lost. Other useful pointers to the nature of the intervening steps are first

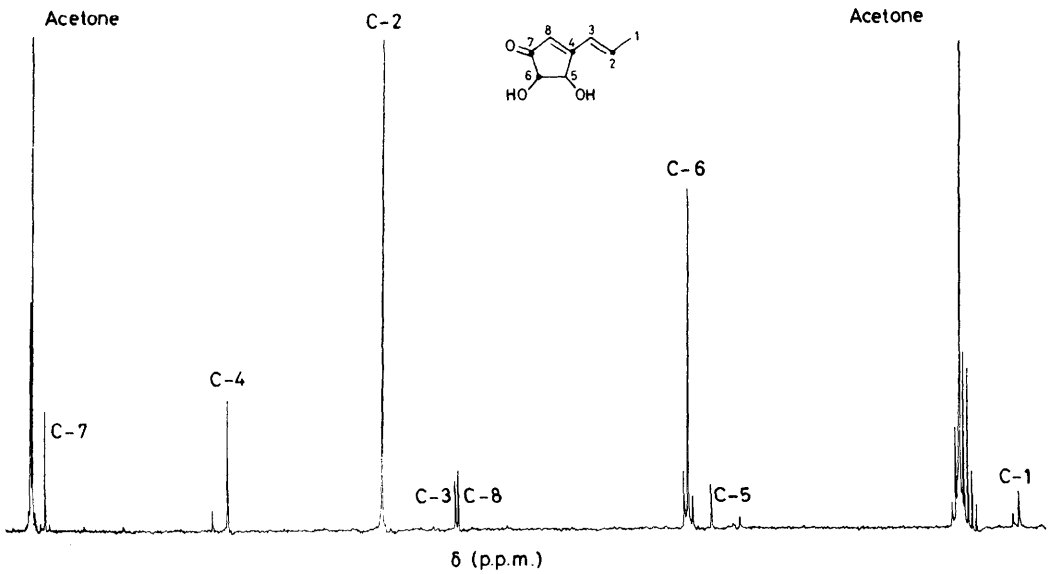


FIGURE 1 Proton-noise decoupled ^{13}C n.m.r. spectrum of terrein enriched with [1-^{13}C]acetate

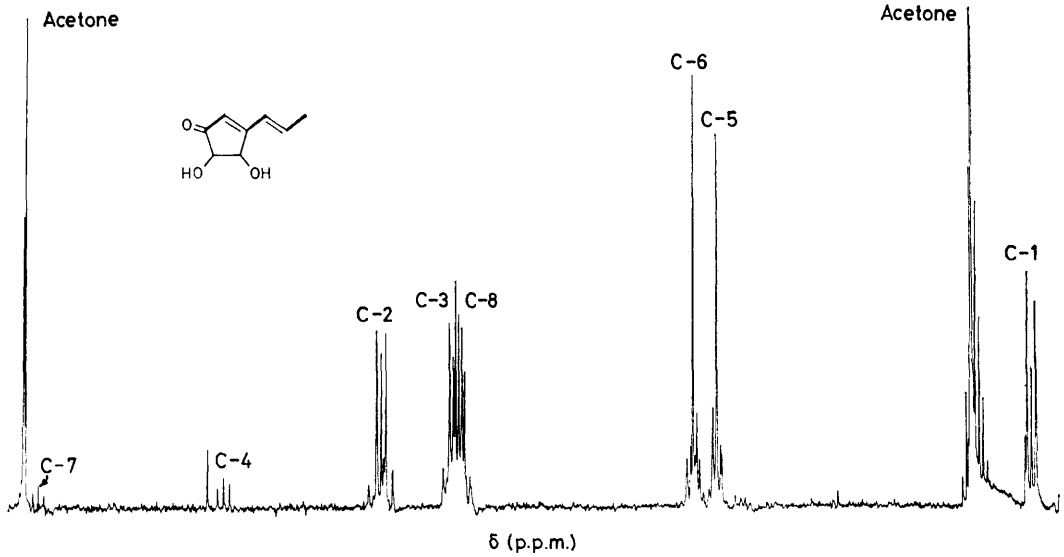
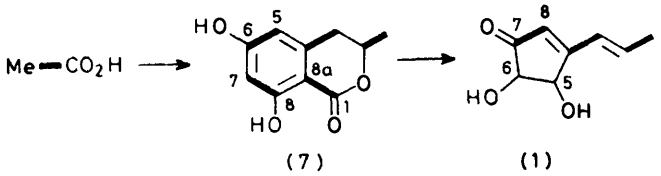


FIGURE 2 Proton-noise decoupled ^{13}C n.m.r. spectrum of terrein enriched with [1,2-$^{13}C_2$]acetate

that symmetrical intermediates can be ruled out and secondly that C-8a becomes hydroxylated.

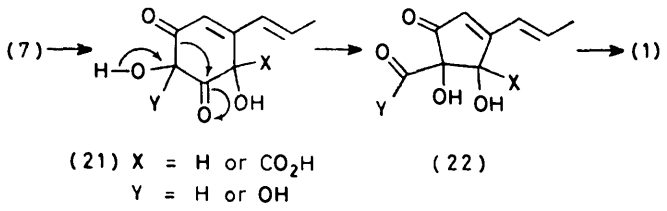
Bearing in mind the chemical precedents for the ring contraction mentioned earlier, we therefore propose that the dihydroisocoumarin (7) is hydroxylated at C-7 and



(7) (1)

SCHEME 4

C-8a to give an intermediate such as (21). This, as shown in Scheme 5, could undergo a ring contraction to (22) from which terrein would be formed by a sequence of chemically plausible steps.



(21) X = H or CO_2H (22)
Y = H or OH

SCHEME 5

A related scheme has been proposed for cryptosporiopsin (18) biosynthesis on the basis of experiments with [1,2-$^{13}C_2$]acetate.[16] The intermediacy of an aromatic precursor in this biosynthesis has yet to be established but if the proposal is correct the two biosyntheses make an interesting contrast, for in one C-7 is lost from the aryl ring whereas C-8 is lost in the other.

EXPERIMENTAL

For general directions see reference 9. M.p.s were determined on a Reichert-Kofler block. Column chromatography was carried out on silica gel (Silicar CC7 Special) or alumina (Woelm neutral, grade II or III). Preparative t.l.c. was carried out on glass plates coated with Merck Kieselgel GF_{254}. Radioactive samples were counted in 7 ml of organic scintillator or aqueous scintillator on a Packard Tri-Carb 3385 instrument and standardised using radiolabelled n-hexadecane as internal standard. Unless otherwise stated, i.r. spectra were recorded for solutions in chloroform on a Perkin-Elmer 257 spectrometer, and n.m.r. spectra for solutions in deuteriochloroform on a Varian HA 100 or HA 100D spectrometer (tetramethylsilane standard). Mass spectra were determined on AEI MS9 and MS12 or MS902 spectrometers employing the direct-inlet system at appropriate temperatures.

3,5-*Bisbenzyloxybenzoic Acid*.—A mixture of methyl 3,5-dihydroxybenzoate [17] (10 g) in dry butanone (130 ml) with benzyl chloride (30 ml) and anhydrous potassium carbonate (50 g) was heated at reflux with stirring for 6 h. After cooling, water (100 ml) and ether (100 ml) were added and the organic layer was separated and washed with 10% sodium hydroxide solution (2 × 50 ml.) The solvents were evaporated off and the residue was heated at reflux with 30% sodium hydroxide solution (60 ml) and ethanol (30 ml) for 2 h. After cooling, water (60 ml) was added and the solution was extracted with ether. The ether layer was extracted with water (2 × 60 ml) and the combined aqueous solutions gave a cream solid on acidification. The *product* was recrystallized from ethanol as needles (14 g, 68%), m.p. 211—212 °C (Found: C, 75.2; H, 5.6. $C_{21}H_{18}O_4$ requires C, 75.4; H, 5.4%); $\nu_{max.}$ 3 000—2 690, 1 690, and 1 600 cm^{-1}; τ[$(CD_3)_2$-CO] 2.66 (10 H, m, Ph), 2.87 (2 H, d, *J* 2 Hz, ArH), 3.13 (1 H, t, *J* 2 Hz, ArH), and 4.88 (4 H, s, CH_2).

3,5-*Bisbenzyloxybenyl Alcohol*.—3,5-Dibenzyloxybenzoic acid (10 g) in dry tetrahydrofuran (THF) (100 ml) was added dropwise to a slurry of lithium aluminium hydride (1 g) in THF (30 ml) with stirring and the mixture was heated at reflux for 5 h. After cooling, water (1 ml) was added cautiously, followed by 15% sodium hydroxide solution (1 ml) and more water (3 ml). The precipitate was removed by filtration and washed with ether (100 ml). The organic solvents were evaporated off to give the *alcohol* which was recrystallised from ether–hexane as needles, (6.5 g, 65%),

m.p. 77—78 °C (Found: C, 78.6; H, 6.3. $C_{21}H_{20}O_3$ requires C, 78.7; H, 6.3%); $\nu_{max.}$ 3 600—3 300 and 1 600 cm^{-1}; τ 2.72 (10 H, s, Ph), 3.50 (3 H, m, ArH), 5.11 (4 H, s, PhCH_2), 5.56 (2 H, s, CH_2OH), and 7.59 (1 H, s, OH).

3,5-*Bisbenzyloxybenzyl Chloride*.—Thionyl chloride (5 ml) was added to a mixture of 3,5-bisbenzyloxybenzyl alcohol (5 g) and pyridine (0.2 ml) in dry ether (50 ml) and the mixture was stirred for 2 h. The excess of thionyl chloride was destroyed with water and the organic layer was washed with 10% sodium hydroxide solution (2 × 40 ml) and water (2 × 40 ml), dried, and evaporated. The *product* was recrystallised from ether as needles (4.0 g, 80%), m.p. 75—76 °C (Found C, 74.5; H, 5.8; Cl, 10.5. $C_{21}H_{19}ClO_2$ requires C, 74.4; H, 5.7; Cl, 10.5%); $\nu_{max.}$ 1 600 cm^{-1}; τ 2.65 (10 H, s, Ph), 3.41 (3 H, m, ArH), 5.03 (4 H, s, PhCH_2), and 5.56 (2 H, s, CH_2Cl).

3,5-*Bisbenzyloxybenzyl Cyanide*.—A mixture of 3,5-bisbenzyloxybenzyl chloride (5 g), potassium cyanide (2.5 g), ethanol (50 ml), and water (25 ml) was heated at reflux for 3 h. The hot solution was poured onto ice (60 g) and extracted with chloroform (3 × 70 ml). The chloroform was dried and evaporated, and the *cyanide* recrystallised from ethyl acetate–hexane as needles, (3.5 g, 70%), m.p. 85—86° (Found: C, 80.2; H, 5.6; N, 4.0. $C_{22}H_{19}NO_2$ requires C, 80.2; H, 5.8; N, 4.2%); $\nu_{max.}$ 2 245 and 1 600 cm^{-1}; τ 2.66 (10 H, s, Ph), 3.49 (3 H, s, ArH), 5.03 (4 H, s, PhCH_2), and 6.47 (2 H, s, CH_2CN).

3,5-*Bisbenzyloxyphenylacetic Acid* (8).—A mixture of 3,5-bisbenzyloxyphenylacetic cyanide (10 g), ethanol (25 ml), and 10% sodium hydroxide solution (100 ml) was heated at reflux for 48 h. After cooling, the solution was acidified and extracted with ether. The ether was dried and evaporated to give the *acid* (6.3 g, 65%), m.p. 107—108 °C (from ethanol) (Found: C, 75.7; H, 5.6. $C_{22}H_{20}O_4$ requires C, 75.8; H, 5.8%); $\nu_{max.}$ (Nujol) 3 000, 1 705, and 1 600 cm^{-1}; τ [$(CD_3)_2CO$] 2.65 (10 H, s, Ph), 3.47 (3 H, s, ArH), 5.00 (4 H, s, PhCH_2), and 6.48 (2 H, s, CH_2CO_2H).

1-(3,5-*Bisbenzyloxyphenyl*)*propan*-2-*one* (9).—Methyl iodide (2 ml) was added dropwise to lithium metal (200 mg) floating on dry ether (50 ml) under nitrogen with stirring. After 1 h the solution of methyl-lithium was added slowly to a solution of 3,5-bisbenzyloxyphenylacetic acid (500 mg) in dry ether (50 ml) under nitrogen. The mixture was stirred at room temperature for 2 h before being added to water (200 ml). The ether layer was separated, washed with 10% sodium hydroxide solution (2 × 50 ml) and water (2 × 50 ml), dried, and evaporated to give a yellow oil. Preparative t.l.c. using dichloromethane as eluant gave the *ketone* as an oil (260 mg, 55%) (Found: M^+, 346.156 $C_{23}H_{22}O_3$ requires M, 346.157); $\nu_{max.}$ 1 710, 1 610, and 1 600 cm^{-1}; τ 2.66 (10 H, s, Ph), 3.56 (3 H, s, ArH), 5.01 (4 H, s, PhCH_2), 6.42 (2 H, ArCH_2), and 7.90 (3 H, s, Me); semicarbazone m.p. 180—181 °C (Found: C, 71.6; H, 6.2; N, 10.3. $C_{24}H_{25}N_3O_3$ requires C, 71.5; H, 6.3; N, 10.4%).

1-(3,5-*Dihydroxyphenyl*)*propan*-2-*one* (2).—A solution of 1-(3,5-bisbenzyloxyphenyl)propan-2-one (30 mg) in ethanol (10 ml) with 5% palladium–charcoal (10 mg) was hydrogenated for 30 min. The charcoal was removed by filtration and the ethanol evaporated to give the product as an oil (17 mg, 90%), b.p. 185—187 °C at 0.05 mmHg (lit.,[18] 185—187 °C at 0.05 mmHg); $\nu_{max.}$ (film) 3 100, 1 720, and 1 600 cm^{-1}; τ[$(CD_3)_2CO$] 3.72 (3 H, s, ArH), 6.43 (2 H, s, CH_2), and 7.88 (3 H, s, Me).

2,5-*Bisbenzyloxybenzoic Acid*.—A mixture of 2,5-dihydroxybenzoic acid (10 g), anhydrous potassium carbonate (75 g), benzyl chloride (30 ml), and butanone (500 ml) was heated at reflux for 16 h with stirring. The preparation was continued as for 3,5-bisbenzyloxybenzoic acid above giving the *product* which was recrystallised from ethanol as needles (14 g, 69%), m.p. 108—109 °C (Found: C, 75.3; H, 5.5. $C_{21}H_{18}O_4$ requires C, 75.4; H, 5.4%); $\nu_{max.}$ 3 280, 1 730, 1 605, and 1 500 cm^{-1}; τ −0.15br (1 H, s, CO_2H), 2.23 (1 H, d, J 3 Hz, 6-H), 2.60 (10 H, s, Ph), 2.94 (2 H, m, 3- and 4-H), 4.80 (2 H, s, PhCH_2), and 4.96 (2 H, s, PhCH_2).

2,5-*Bisbenzyloxybenzyl Alcohol*.—Prepared from 2,5-bisbenzyloxybenzoic acid (10 g) by the method described above for 3,5-bisbenzyloxybenzyl alcohol, the *product* recrystallised from chloroform–hexane as needles (7.7 g, 80%), m.p. 56—57 °C (Found: C, 78.5; H, 6.1. $C_{21}H_{20}O_3$ requires C, 78.5; H, 6.3%); $\nu_{max.}$ 3 600—3 300, 1 600, 1 580, and 1 500 cm^{-1}; τ 2.68 (10 H, s, Ph), 3.02 (1 H, m, 6-H), 3.21 (2 H, m, 3- and 4-H), 5.02 (2 H, s, PhCH_2), 5.05 (2 H, s, PhCH_2), 5.36 (2 H, s, CH_2OH), and 7.64 (1 H, s, OH).

2,5-*Bisbenzyloxybenzyl Chloride*.—Prepared from 2,5-bisbenzyloxybenzyl alcohol, the *product* recrystallised from ether as needles (4.7 g, 90%), m.p. 82—83 °C (Found: C, 74.2; H, 5.7; Cl, 10.6; $C_{21}H_{19}ClO_2$ requires C, 74.4; H, 5.7; Cl, 10.5%); $\nu_{max.}$ 1 600, 1 580, and 1 500 cm^{-1}; τ 2.65 (10 H, s, Ph), 2.99 (1 H, m, ArH), 3.19 (2 H, m, ArH), 4.99 (2 H, s, PhCH_2), 5.05 (2 H, s, PhCH_2), and 5.48 (2 H, s, CH_2Cl).

2,5-*Bisbenzyloxybenzyl Cyanide*.—Prepared from 2,5-bisbenzyloxybenzyl chloride (5 g) by the method used for 3,5-bisbenzyloxybenzyl cyanide, the *product* was recrystallised from ethyl acetate–hexane as needles, (3.2 g, 65%), m.p. 94—95 °C (Found: C, 80.0; H, 5.6; N, 3.9. $C_{22}H_{19}NO_2$ requires C, 80.2; H, 5.8; N,4 .2%); $\nu_{max.}$ 2 245, 1 595, and 1 500 cm^{-1}; τ 2.74 (10 H, s, Ph), 3.06 (1 H, s, ArH), 3.28 (2 H, s, ArH), 5.11 (4 H, s, PhCH_2), and 6.48 (2 H, s, CH_2CN).

2,5-*Bisbenzyloxyphenylacetic Acid*.—Prepared from 2,5-dibenzyl cyanide (3 g) by the method used for 3,5-bisbenzyloxyphenylacetic acid, the *product* recrystallised from aqueous ethanol as needles (2.6 g, 75%), m.p. 105—106 °C (Found: C, 76.0; H, 5.6. $C_{22}H_{20}O_4$ requires C, 75.6; H, 5.8%); $\nu_{max.}$ 3 000, 1 710, 1 600, 1 590, and 1 500 cm^{-1}; τ −0.20 (1 H, s, CO_2H), 2.65 (10 H, s, Ph), 3.13 (1 H, m, ArH), 3.19 (2 H, m, ArH), 5.02 (4 H, s, PhCH_2), and 6.34 (2 H, s, CH_2).

1-(2,5-*Bisbenzyloxyphenyl*)*propan*-2-*one*.—Prepared from 2,5-bisbenzyloxyphenylacetic acid (500 mg) by the method used for 1-(3,5-dibenzyloxyphenyl)propan-2-one, the *product* recrystallised from ether–hexane as needles (370 mg, 74%), m.p. 63—64 °C (Found: C, 79.8; H, 6.4. $C_{23}H_{22}O_3$ requires C, 79.7; H, 6.4%); $\nu_{max.}$ 1 705, 1 600, 1 580, and 1 500 cm^{-1}; τ 2.69 (10 H, s, Ph), 3.22 (3 H, s, ArH), 5.04 (4 H, s, PhCH_2), 6.38 (2 H, s, CH_2COCH_3), and 7.93 (3 H, s, Me).

1-(2,5-*Dihydroxyphenyl*)*propan*-2-*one* (3).—A solution of 1-(2,5-bisbenzyloxyphenyl)propan-2-one (290 mg) in ethanol (25 ml) with 10% palladium–charcoal (50 mg) was hydrogenated at 1 atm for 3 h (uptake 52 ml). The catalyst was removed and the alcohol evaporated to give the *product* which recrystallised from benzene (133 mg, 95%), m.p. 81—83° (lit.,[19] 80—83 °C).

3-*Benzyloxyphenylacetic Acid*.—A mixture of 3-hydroxyphenylacetic acid (6.12 g), anhydrous potassium carbonate (52 g), benzyl chloride (25 g), and butanone (150 ml) was heated at reflux with stirring for 6 h. After cooling, water (300 ml) was added and the mixture was extracted with ether (2 × 200 ml). The ether was washed with water

(100 ml), 10% sodium hydroxide solution (100 ml) and water (100 ml), dried and evaporated to give a pale yellow oil. This was mixed with 10% sodium hydroxide solution (100 ml) and ethanol (30 ml) and heated at reflux for 2 h. After cooling, the mixture was extracted with ether (50 ml), and the ether layer was extracted with water (50 ml). The aqueous layers were acidified and the *product* collected and recrystallised from aqueous ethanol as needles (7.0 g, 73%), m.p. 121—122 °C (Found: C, 74.6; H, 5.8. $C_{15}H_{14}O_3$ requires C, 74.4; H, 5.8%); $\nu_{max.}$ 3 000, 1 710, and 1 600 cm^{-1}; τ −0.10 (1 H, s, CO_2H), 2.66 (5 H, s, Ph), 3.11 (2 H, m, ArH), 3.17 (2 H, m, ArH), 4.99 (2 H, s, PhCH_2), and 6.42 (2 H, s, CH_2CO_2H).

1-(3-*Benzyloxyphenyl*)*propan*-2-*one*.—Prepared from 3-benzyloxyphenylacetic acid (370 mg) by the method used for 1-(3,5-bisbenzyloxyphenyl)propan-2-one, and purified by preparative t.l.c. using dichloromethane as eluant, the *ketone* was obtained as an oil (280 mg, 75%); $\nu_{max.}$ 1 710 and 1 600 cm^{-1}; τ 2.67 (5 H, s, Ph), 3.20 4 H, m, ArH), 5.01 (2 H, s, PhCH_2), 6.43 (2 H, s, CH_2COCH_3), and 7.96 (3 H, s, CH_3); semicarbazone m.p. 155—156 °C (Found C, 68.8; H, 6.3; N, 14.1. $C_{17}H_{19}N_3O_2$ requires C, 68.7; H, 6.4; N, 14.1%).

1-(3-*Hydroxyphenyl*)*propan*-2-*one*.—A solution of the foregoing ketone (550 mg) in ethanol (25 ml) with 10% palladium–charcoal (100 mg) and concentrated hydrochloric acid (0.05 ml) was hydrogenated at room temperature and 1 atm (uptake 75 ml hydrogen). The catalyst was removed by filtration and the ethanol was evaporated off to give the *product* (300 mg, 90%) (Found: M^+, 150.068. $C_9H_{10}O_2$ requires M, 150.068); $\nu_{max.}$ (film) 3 300, 1 700, 1 600, and 1 460 cm^{-1}; τ 2.5 (1 H, s, OH), 2.8—3.3 (4 H, m, ArH), 6.40 (2 H, s, CH_2), and 7.98 (3 H, s, CH_3).

1-(3-*Benzyloxyphenyl*)*propan*-2-*ol*.—Sodium borohydride (2.5 g) was added to a solution of 1-(3-benzyloxyphenyl)-propan-2-one (3.0 g) in ethanol (70 ml) and stirred for 2 h. The mixture was diluted with water (250 ml) and extracted with ether (3 × 100 ml). Evaporation of the dried ethereal extract gave the *alcohol* as an oil (2.4 g, 80%) (Found: M^+, 242.130. $C_{16}H_{18}O_2$ requires M, 242.131); $\nu_{max.}$ 3 350, 1 605, 1 585, and 1 490 cm^{-1}; τ 2 64 (5 H, s, Ph), 3.15 (4 H, m, ArH), 4.96 (2 H, s, PhCH_2), 5.98 (1 H, m, J 6 Hz, CHOH), 7.30 (2 H, m, CH_2), 8.40 (1 H, s, OH), and 8.78 (3 H, d, J 6 Hz, Me).

1-(3-*Benzyloxyphenyl*)-2-*chloropropane*.—Thionyl chloride (5 ml) was added to a stirred solution of 1-(3-benzyloxyphenyl)propan-2-ol (2.4 g) in dry ether (50 ml) containing dry pyridine (0.1 ml). After 1 h, water (50 ml) was added cautiously and the aqueous layer was extracted with ether (50 ml). The ethereal solution was washed with water (2 × 50 ml), 10% (w/v) sodium hydroxide solution (2 × 50 ml) and water (50 ml), dried, and evaporated to give the *chloropropane* as an oil (2.2 g, 85%) (Found: M^+, 260.098. $C_{16}H_{17}ClO$ requires M, 260.097); $\nu_{max.}$ 1 600, 1 485, and 1 260 cm^{-1}; τ 2.64 (5 H, s, Ph), 2.75—3.15 (4 H, m, ArH), 4.97 (2 H, s, PhCH_2), and 5.72 (1 H, m, J 7 Hz, Me).

2-*Chloro*-1-(3-*hydroxyphenyl*)*propane*.—1-(3-Benzyloxyphenyl)-2-chloropropane (1.5 g) in ethanol (30 ml) containing concentrated hydrochloric acid (0.1 ml) was hydrogenated over 5% palladium–charcoal (100 mg) at 1 atm for 1 h. The catalyst was removed and the alcohol evaporated off. A solution of the residue in ethyl acetate (50 ml) was extracted with 10% (w/v) sodium hydroxide solution (2 × 50 ml). The acidified aqueous extracts were extracted in turn with ethyl acetate (3 × 50 ml). The latter solutions were dried and evaporated to give the *phenol* as an oil (800 mg, 78%) (Found: M^+, 170.049. $C_9H_{11}ClO$ requires M, 170.050); $\nu_{max.}$ 3 400, 1 600, 1 595, 1 450, and 1 260 cm^{-1}; τ 2.7—3.4 (4 H, m, ArH), 4.24 (1 H, s, OH), 5.86 (1 H, m, CHCl), 7.03 (2 H, m, CH_2), and 8.52 (3 H, d, J 6 Hz, Me).

1-(3-*Hydroxyphenyl*)*prop*-1-*ene* (5).—A solution of 2-chloro-1-(3-hydroxyphenyl)propane (800 mg) in 0.5M-ethanolic potassium hydroxide (50 ml) was heated at reflux for 3 h. After cooling, the reaction mixture was evaporated to small volume, acidified, and extracted into ethyl acetate (2 × 50 ml). The dried organic layer was evaporated and the residue, after preparative t.l.c. using dichloromethane as eluant, gave the *alkene* as an oil (485 mg, 77%) (Found: M^+, 134.073. $C_9H_{10}O$ requires M, 134.073); $\nu_{max.}$ 3 590, 1 600, 1 490, and 1 160 cm^{-1}; τ 2.7—3.4 (4 H, m, ArH), 3.4—4.2 (2 H, m, olefinic), 5.10 (1 H, s, OH), and 8.17 (3 H, d, J 6 Hz, Me).

1-(3,5-*Bisbenzyloxyphenyl*)*propan*-2-*ol* (10).—A solution of 1-(3,5-bisbenzyloxyphenyl)propan-2-one (1.2 g) in ethanol (50 ml) was stirred for 2 h with sodium borohydride (1 g), then diluted with water (450 ml) and extracted with ether (3 × 50 ml). The dried ethereal solution was evaporated to give an oil which was purified by chromatography on silica gel using dichloromethane as eluant to give the *alcohol* as an oil (1.1 g, 90%) (Found: M^+, 348.172. $C_{23}H_{24}O_3$ requires M, 348.173); $\nu_{max.}$ 3 400, 1 600, and 1 500 cm^{-1}; τ 2.66 (10 H, s, Ph), 3.54 (3 H, s, ArH), 5.01 (4 H, s, PhCH_2), 6.09 (1 H, m, CHOH), 7.35 (2 H, m, CH_2), 8.40 (1 H, s, OH), and 8.80 (3 H, d, J 6 Hz, Me).

1-(3,5-*Bisbenzyloxyphenyl*)-2-*chloropropane* (11).—A solution of phosphorus pentachloride (3.5 g) and 1-(3,5-bisbenzyloxyphenyl)propan-2-ol (3.48 g) in dry ether (100 ml) was stirred for 30 min. Water (50 ml) was then added cautiously. The ethereal layer was washed with water (50 ml), 10% (w/v) sodium hydroxide solution (2 × 50 ml), and water (50 ml), dried, and evaporated to give an oil which was purified by chromatography on silica gel using dichloromethane as eluant, to give the *chloropropane* (3.05 g, 83%) (Found: M^+, 368.136. $C_{23}H_{23}ClO_2$ requires M, 368.136); $\nu_{max.}$ 1 590, 1 450, and 1 155 cm^{-1}; τ 2.70 (10 H, s, Ph), 3.51 (3 H, m, ArH), 5.03 (4 H, s, PhCH_2), 5.65 (1 H, m, CHCl), 6.8—7.2 (2 H, m, CH_2), and 8.56 (3 H, d, J 6 Hz, Me).

1-(3,5-*Dihydroxyphenyl*)-2-*chloropropane* (12).—Prepared from 1-(3,5-bisbenzyloxyphenyl)-2-chloropropane (650 mg) by the method described above for 1-(3-hydroxyphenyl)-2-chloropropane, the *phenol* was purified by preparative t.l.c. using dichloromethane as eluant (280 mg, 85%) (Found: M^+, 186.045. $C_9H_{11}ClO_2$ requires M, 186.045); $\nu_{max.}$ 3 250, 1 700, 1 600, and 1 150 cm^{-1}; τ [$(CD_3)_2CO$] 1.92 (2 H, s, OH), 3.72 (3 H, s, ArH), 3.70 (1 H, m, CHCl), 7.08 (2 H, m, CH_2), and 8.48 (3 H, d, J 7 Hz, Me).

1-(3,5-*Dihydroxyphenyl*)*prop*-1-*ene* (4).—A solution of 1-(3,5-dihydroxyphenyl)-2-chloropropane (280 mg) in 0.5M-ethanolic potassium hydroxide (40 ml) was heated at reflux for 3 h, cooled, and evaporated to small volume. The residue was treated with dilute hydrochloric acid (50 ml) and then extracted into ethyl acetate (3 × 50 ml). The combined extracts were dried and evaporated and the product was purified by preparative t.l.c. using dichloromethane as eluant to give the *alkene* as an oil (160 mg, 70%) (Found: M^+, 150.068. $C_9H_{10}O_2$ requires M, 150.068); $\nu_{max.}$ 3 350, 1 690, 1 600, and 1 440 cm^{-1}; τ [$(CD_3)_2CO$] 2.06 (2 H, s, OH), 3.65 (3 H, d, J 2 Hz, ArH), 3.83 (2 H, m, olefinic), and 8.21 (3 H, d, J 5 Hz, Me).

6,8-*Dihydroxy*-3-*methyliscoumarin* (6).—6,8-Dimethoxy-3-

methylisocoumarin [4] (510 mg) in dry dichloromethane (40 ml) was cooled to −70 °C and boron tribromide (2 ml) was added; the stirred solution was allowed to warm to room temperature over 16 h. Ether (60 ml) was added and the solution was washed with water (50 ml), dried, and evaporated. Sublimation of the residue (180 °C at 0.05 mmHg) gave the product as a powder (380 mg, 85%), m.p. 250—252 °C (lit.,[20] 250—253 °C) (Found: C, 62.6; H, 4.2%; M^+, 192.042. Calc. for $C_{10}H_8O_4$: C, 62.5; H, 4.2%; M, 192.042); $\nu_{max.}$ (Nujol) 3 200, 1 670, and 1 620 cm^{-1}; τ [$(CD_3)_2CO$] 3.64 (3 H, s, ArH) and 7.78 (3 H, s, Me).

3,4-*Dihydro*-6,8-*dimethoxy*-3-*methyliscoumarin* (16).—6,8-Dimethoxy-3-methylisocoumarin [8] (120 mg) in dry ethyl acetate (20 ml) was hydrogenated at 1 atm in the presence of 10% palladium–charcoal. When the uptake of hydrogen ceased (uptake 20 ml) the catalyst was removed by filtration and the solvent was evaporated off to give a powder which recrystallised from ethyl acetate–hexane as prisms (110 mg, 90%), m.p. 125—126 °C (lit.,[21] 125—126 °C); $\nu_{max.}$ 1 710 and 1 600 cm^{-1}; τ 3.62 (1 H, m, J 2 Hz, ArH), 3.70 (1 H, m, J 2 Hz, ArH), 5.51 (1 H, m, J 7 Hz, 3-H), 6.13 (3 H, s, OMe), 6.18 (3 H, s, OMe), 7.19 (2 H, d, J 7 Hz, CH_2), and 8.56 (3 H, d, J 7 Hz, Me).

3,4-*Dihydro*-6,8-*dihydroxy*-3-*methyliscoumarin* (7).—A solution of 3,4-dihydro-6,8-dimethoxy-3-methylisocoumarin (110 mg) in dry dichloromethane (10 ml) was cooled to −70 °C and boron tribromide (0.5 ml) was added. The mixture was allowed to warm to room temperature over 16 h. The solution was then diluted with ether (20 ml) and washed with water (3 × 20 ml). The ethereal solution was dried and evaporated to give a powder which was recrystallised from acetone–hexane to give the product as needles (80 mg, 82%), m.p. 214—215 °C (lit.,[9] 214—215 °C); $\nu_{max.}$ 3 400 and 1 650 cm^{-1}; τ [$(CD_3)_2CO$] 3.74 (2 H, s, ArH), 5.21 (1 H, d, q, J 6 and 2 Hz, 3-H), 7.08 (1 H, s, 4-H), 7.19 (1 H, d, J 2 Hz, 4-H), and 8.53 (3 H, d, J 6 Hz, Me).

Kuhn–Roth Oxidation of Terrein.—Terrein (50 mg) was heated at 100 °C with 5M-chromic acid (10 ml) and concentrated sulphuric acid (2 ml) for 1.5 h. After cooling the product was steam-distilled (400 ml collected). The distillate was boiled to remove carbon dioxide, neutralised with 0.05M-sodium hydroxide solution (phenolphthalein indicator) and then evaporated to dryness. A solution of the residue with *p*-bromophenacyl bromide (50 mg) in water (1 ml) and ethanol (9 ml) was heated at reflux for 2 h. The solution was then concentrated to small volume and the residue was partitioned between ether (10 ml) and water (10 ml). The aqueous layer was extracted with more ether (2 × 10 ml) and the combined ethereal solutions were dried and evaporated. The residue was purified by preparative t.l.c. using ethyl acetate–benzene (1 : 10 v/v) as eluant to give the *p*-bromophenyl acetate (30 mg), m.p. 85—86 °C [from light petroleum (b.p. 60—80 °C)] (lit.,[22] 86 °C).

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REFERENCES

[1] Preliminary account, *J. Chem. Soc., Chem. Commun.*, 1975, 380.
[2] H. Raistrick and G. Smith, *Biochem. J.*, 1935, **29**, 606.
[3] P. W. Clutterbuck, H. Raistrick, and F. Reuter, *Biochem. J.*, 1937, **31**, 987.
[4] J. F. Grove, *J. Chem. Soc.*, 1954, 4693.
[5] D. H. R. Barton and E. Miller, *J. Chem. Soc.*,1955, 1028.
[6] J. Auerbach and S. M. Weinreb, *J. Chem. Soc., Chem. Commun.*, 1974, 298.
[7] A. J. Birch, A. Cassera, and A. R. Jones, *Chem. Commun.*, 1965, 167.
[8] M. Biollaz, G. Buchi, and G. Milne, *J. Am. Chem. Soc.*, 1968, **90**, 5017, 5019; *ibid.*, 1970, **92**, 1035.
[9] R. H. Carter, R. M. Colyer, R. A. Hill, and J. Staunton, *J. Chem. Soc.*, 1976, 1438.
[10] R. F. Curtis, P. C. Harries, C. H. Hassal, J. D. Levi, and D. M. Phillips, *J. Chem. Soc. C.*, 1966, 168.
[11] C. J. Moye and S. Sternhell, *Aust. J. Chem.*, 1966, **19**, 2107; A. W. Burgstahler, T. B. Lewis, and M. C. Abdet-Rahman, *J. Org. Chem.*, 1966, **31**, 3516.
[12] G. M. Strunz, A. S. Court, and J. Kamlossy, *Tetrahedron Lett.*, 1969, 3613; G. M. Strunz and A. S. Court, *J. Am. Chem. Soc.*, 1973, **95**, 3000.
[13] E. Collins and P. U. R. Shannon, *J. Chem. Soc., Perkin Trans. 1*, 1973, 419.
[14] T. J. Simpson, *Chem. Soc. Rev.*, 1975, **4**, 497.
[15] B. Birdsall, N. J. M. Birdsall, and J. Feeney, *J. Chem. Soc., Chem. Commun.*, 1972, 316.
[16] J. S. E. Holker and K. Young, *J. Chem. Soc., Chem. Commun.*, 1975, 525.
[17] J. H. Birkinshaw and A. Bracken, *J. Chem. Soc.*, 1942, 368.
[18] H. Stetter and R. Hesse, *Monatsh. Chem.*, 1967, **98**, 755.
[19] J. M. Bruce, D. Creed, and K. Davies, *J. Chem. Soc. C.*, 1971, 3749.
[20] T. Money, F. W. Camer, G. R. B. Webster, I. G. Wright, and A. I. Scott, *Tetrahedron*, 1967, **23**, 3435.
[21] W. J. Mitrahren and L. A. Mitscher, *J. Org. Chem.*, 1968, **33**, 1579.
[22] A. I. Vogel, 'Practical Organic Chemistry,' Longmans, London, 1948.