

Finally it is worth pointing out that the ratios $k_{M,L}^{MI}/k^{ML}$ agree nicely with the equilibrium constants measured potentiometrically (Table 3).

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208. Biosynthesis of Cytochalasins. Part 4. The Mode of Incorporation of Common Naturally-Occurring Carboxylic Acids into Cytochalasin D¹⁾

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(13. VI. 75)

Summary. Utilization of sodium [1-¹⁴C]-, [2-¹⁴C]-, and [1,2-¹³C]-acetates, [1-¹⁴C]-, [1-¹³C]-, or [2-¹⁴C]-propionates, [1-¹⁴C]- or [2-¹⁴C]-malonates, of [1-¹⁴C]- or of [1-¹³C]-myristic acid, or of [1-¹⁴C]- and [1-¹³C]-palmitic acid in the biosynthesis of cytochalasin D (I) by *Zygosporium masonii* was determined by degradation studies or by carbon magnetic resonance spectroscopy. The precursors were incorporated primarily via the acetate-malonate pathway to generate I from nine intact acetate units, eight of which are coupled in a head to tail fashion to form the C₁₈-polyketide moiety.

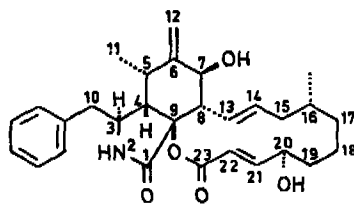
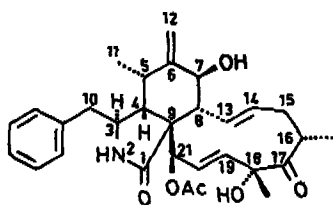
1. Introduction. - The nineteen cytochalasins isolated since 1966 comprise a class of microbial metabolites with great potential as tools in cell biology and medicine.

¹⁾ Part 3 see [1].

A multitude of reports on their physiological activity have appeared, and the number of cytochalasins has almost doubled since their chemistry [2] and biological action [3] were reviewed. Revision of the structures [4] of cytochalasins E and F was followed by the isolation of desoxaphomin [5], protophomin and proxiphomin [6], chaetoglobosins A, B and C [7], kodocytchalasin and its desacetyl derivative [8], and cytochalasin G [9^a].

These perhydro-isoindolones all possess a macrocyclic ring and some have in common a number of unusual biological effects which are very dependent on structure. Reversible inhibition of cytoplasmic cleavage resulting in polynucleate cells, inhibition of cell movement, and nuclear extrusion are among a few of the most striking characteristics [11]. The latter effect has been used to separate viable nuclei (karyoplasts) from their cytoplasm [12]. Some cytochalasins affect fungal growth [13], phagocytosis [14], platelet aggregation and clot retraction [15], glucose transport [16], and parathyroid secretion [17]. So far only cytochalasins A, B, D and E have been studied biologically in detail. Japanese workers have tested structure-activity relationships of cytochalasin D (zygospurin A) (1) and its derivatives in an attempt to lower toxicity without losing strong tumor-inhibiting properties [18]. Recent cytological studies with chaetoglobosins indicated high activity despite replacement of the usual phenyl group with an indolyl moiety [19].

Investigation of the biosynthesis of cytochalasin B (phomin) (2) by *Phoma* sp. (S 298) demonstrated that phenylalanine is attached to an acetate-malonate derived C₁₈-polyketide element which contains two C₁ substituents originating from the methyl of methionine [2] [20]. Analogously, *Zygosporium masonii* HUGHES (*Fungi imperfecti*) produces cytochalasin D (1) from phenylalanine, acetate, and three units of methionine [21]. Complete assignment of the ¹³C-NMR. spectra of cytochalasins D (1) and B (2), followed by incorporation of ¹³C-labelled acetate elucidated the polyketide labelling pattern [1]. We now report on the utilization of a series of common carboxylic acids in the biosynthesis of cytochalasin D (1) by *Z. masonii*.



2. Incorporation of Acetate. - Prior to experiments with ¹³C-labelled precursors, fermentations of *Z. masonii* with sodium [1-¹⁴C]-acetate and [2-¹⁴C]-acetate resp. were completed, and showed absolute incorporation rates of 2.6% and 1.4% resp. into cytochalasin D (1) (Table 1). To ascertain the labelling pattern and to provide a chemical basis for such localizations in trials with other precursors, four degradation sequences were carried out.

^a) Cytochalasin B (2) has been recently re-isolated from tomatoes contaminated with *Hormiscium* sp. [10].

Table 1. Incorporation of ^{14}C -Precursors into Cytochalasin D (1)

Precursor Compound Incorporated	Cytochalasin D (1)					
	Activity Total (mCi)	Specific (mCi/ mmol)	Activity dpm/mg	dpm/mmol	Incorporation Rate (%) Absolute	Specific
sodium [1- ^{14}C]-acetate	0.25	58.0	22700	$115 \cdot 10^5$	2.58	$89.3 \cdot 10^{-4}$
sodium [2- ^{14}C]-acetate	0.50	60.0	10500	$53.0 \cdot 10^5$	1.38	$39.8 \cdot 10^{-4}$
sodium [1- ^{14}C]-malonate	0.25	6.9	425	$2.15 \cdot 10^5$	0.077	$14.0 \cdot 10^{-4}$
sodium [2- ^{14}C]-malonate	0.05	10.3	268	$1.36 \cdot 10^5$	0.24	$5.95 \cdot 10^{-4}$
diethyl [2- ^{14}C]-malonate	0.05	20.0	31800	$160 \cdot 10^5$	1.21	$363 \cdot 10^{-4}$
sodium [1- ^{14}C]-propionate	0.05	57.0	495	$2.51 \cdot 10^5$	0.068	$1.98 \cdot 10^{-4}$
sodium [2- ^{14}C]-propionate	0.25	15.6	7410	$37.7 \cdot 10^5$	1.80	$108 \cdot 10^{-4}$
sodium [^{14}C]-formate	0.10	61.3	2230	$11.1 \cdot 10^5$	0.095	$8.30 \cdot 10^{-4}$
sodium [1- ^{14}C]-butyrate	0.10	25.8	12300	$62.0 \cdot 10^5$	0.24	$109 \cdot 10^{-4}$
[1- ^{14}C]-myristic acid ^{a)}	0.05	45.0	5210	$26.4 \cdot 10^5$	0.58	$23.9 \cdot 10^{-4}$
[1- ^{14}C]-palmitic acid ^{a)}	0.05	57.9	510	$2.58 \cdot 10^5$	0.082	$2.00 \cdot 10^{-4}$
sodium [5- ^{14}C]-mevalonate	0.05	12.3	77	$0.39 \cdot 10^5$	0.009	$1.43 \cdot 10^{-4}$

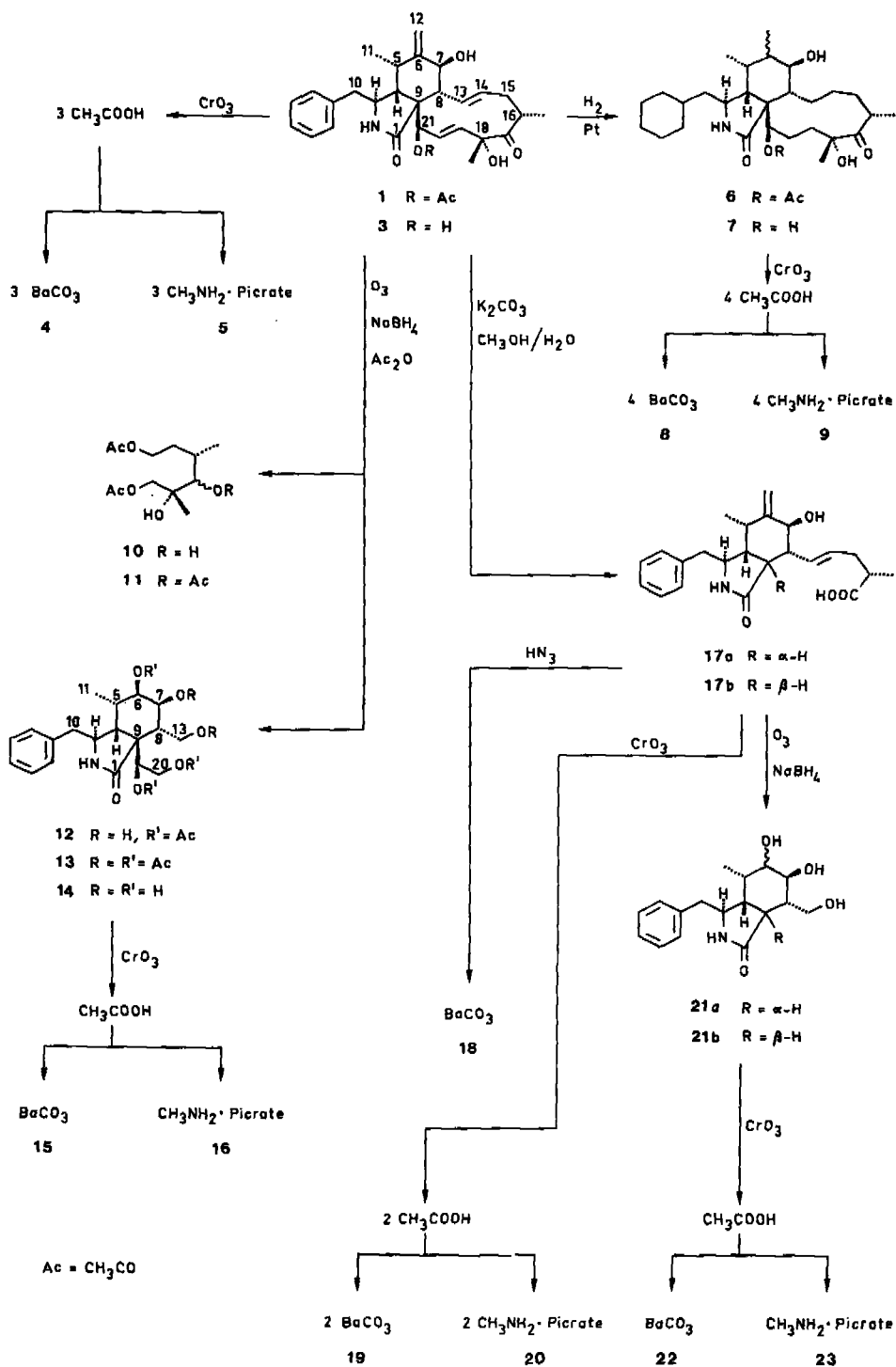
^{a)} See Exper. Part, 2.

Radioactive cytochalasin D (1), obtained after fermentation, was diluted with inactive material³⁾ and hydrolysed under mild conditions to give desacetylcytochalasin D (3) [21] [22]. *Kuhn-Roth* degradation [23] of 3 produced three equivalents of acetic acid (one for each methyl group) which were further cleaved by the *Schmidt* reaction [24] to carbon dioxide (isolated as BaCO_3 (4)) and methylamine (isolated as the picrate (5)). This permitted determination of activity for the O-acetyl group as well as that for C(5), C(16), and C(18) combined, and for C(11), C(16) methyl, and C(18) methyl combined.

In the second degradation, cytochalasin D (1) was first hydrogenated to epimeric dodecahydrocytochalasins (6) which were hydrolyzed to their desacetyl derivatives 7 before *Kuhn-Roth* oxidation [21] [22]. The resulting four equivalents of acetic acid were subjected to the *Schmidt* reaction as before. In addition to providing an independent determination of labelling for the O-acetyl group, this sequence also furnished the activity at C(6) and C(12) by comparison with the first degradation series.

Ozonolysis of cytochalasin D (1), reductive cleavage of the ozonide by sodium borohydride and acetylation produced 1,6-diacetoxy-2,4-dimethyl-hexan-2,3-diol (10), 1,3,6-triacetoxy-2,4-dimethyl-hexan-2-ol (11) and the lactams 12 and 13.

³⁾ We are indebted to Dr. E. Härrä and Mr. J. Hofmann and J. Bianchi, Sandoz AG., Basle, for 500 liter and 100 liter fermentations of *Z. masonii*.



Extensive NMR.-decoupling experiments and comparison of observed proton coupling constants ($J_{5,6} = 6$ Hz; $J_{6,7} = 6$ Hz; $J_{7,8} = 12$ Hz) with those calculated by consideration of stable conformations of **12** and **13**, allowed assignment of the stereochemistry. Hydrolysis of compounds **12** and **13** gave the same lactam pentol **14**, which was analyzed by the usual *Kuhn-Roth* and *Schmidt* sequence to determine activity at C(5) and C(11).

In the final series of cleavages, drastic hydrolysis of cytochalasin D (**1**) yielded a separable mixture of epimeric acids **17a** and **17b**. *Schmidt* degradation of **17a** and **17b** and fixation of evolved the carbon dioxide with barium hydroxide localized the label at C(17) of **1**. *Kuhn-Roth* oxidation of **17a** and **17b** and *Schmidt* cleavage of the resulting two equivalents of acetic acid yielded the activities of C(16) and of its methyl substituent by comparison with the same sequence on lactam pentol **14**. Since combined radioactivity values for the methyl groups of cytochalasin D (**1**) and for their adjacent atoms were known from the first degradation, the labelling at C(18) and at its methyl group could be obtained by difference. Ozonolysis of **17a** and **17b** followed by reduction gave epimeric lactam triols **21a** and **21b** resp. The usual *Kuhn-Roth* and *Schmidt* route offered independent determinations of C(11) and C(5). A combined value for C(14) and C(15) could be calculated from the activity difference between lactams **17** and **21** since labelling at C(17), C(16), and the C(16) methyl had been previously elucidated. With the radioactivities of C₁₄-C₁₈ including attached methyl groups known, a determination could now be made for C(20) and C(21) combined

Table 2. Activity of Products from Degradation of Cytochalasin D (**1**)

	[1- ¹⁴ C]-Acetate		[2- ¹⁴ C]-Acetate		[2- ¹⁴ C]-Propionate	
	dpm/mg	%	dpm/mg	%	dpm/mg	%
1 ^{b)}	2260	100.0	2100	100.0	2090	100.0
3	2050	83.3	1980	86.5	1970	86.7
4	192	9.9	315	17.4	180	10.0
5	30	2.0	110	8.2	0	0.0
6	2140	97.0	1980	96.6	1997	97.8
7	2140	89.0	1920	86.1	1923	86.7
8	148	10.2	333	24.7	130	9.7
9	30	2.7	95	9.3	0	0.0
10	1410	32.3	1450	35.6	1410	32.4
11	1290	34.3			1330	38.0
12	1355	57.9			1230	57.0
13	1160	58.2	1200	64.5	1070	58.0
14	1590	50.6	1550	53.1	1510	51.9
15	505	8.7	85	1.6		
16	40	0.9	230	5.7		
17 ^{a)}	1880	62.9	1790	64.4	1810	65.4
18 ^{a)}	620	10.7	115	2.1	470	8.9
19	258	8.8	214	8.0	248	9.2
20	25	0.5	170	8.3	17	0.2
21 ^{a)}	1450	38.7	1470	42.2	1477	42.4
22	502	8.6	85	1.6	444	8.3
23	10	0.2	230	5.7	18	0.4

^{b)} Radioactive cytochalasin D (**1**), obtained by fermentation, was diluted with inactive material prior to degradation; the activities are of the diluted substance.

^{a)} Average values from separate degradations of both epimers.

and for C(19) by considering the difference between lactams **14** and **21**. Activities of the degradation products are listed in Table 2.

Using these procedures, the distribution of radioactivity from incorporations of sodium $[1-^{14}\text{C}]$ -acetate and $[2-^{14}\text{C}]$ -acetate resp. could be summarized as shown in Fig. 1. Subsequent feeding of the corresponding ^{13}C -labelled acetates confirmed these results and established the alternating pattern of labelling in the C_{16} -polyketide moiety [1]. Such a pattern can arise either from a single polyketide chain **A**, or from multiple chains **B** or **C** which are first joined and then cleaved (Fig. 2). Cytochalasin D (**1**) possesses arrangement type **A** or **C** as shown by the mode of incorporation of doubly labelled sodium $[1,2-^{13}\text{C}]$ -acetate (Fig. 3)⁷⁾.

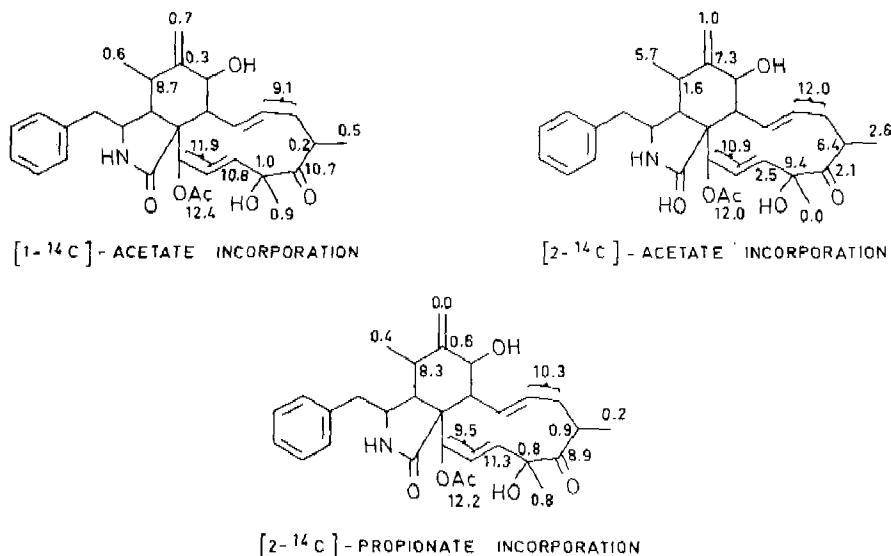


Fig. 1. Distribution of Radioactivity in Percent as Determined by Degradation; Cytochalasin D = 100%

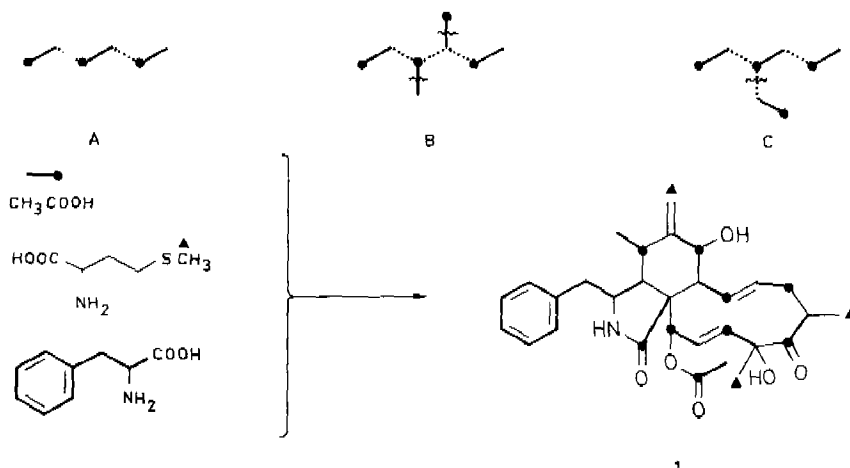


Fig. 2. Distribution Pattern of Biosynthetic Units in Cytochalasin D

⁷⁾ We are grateful to Prof. K. Nakanishi and Drs. P. Solomon and I. Miura of Columbia University for the measurement and analysis of these spectra.

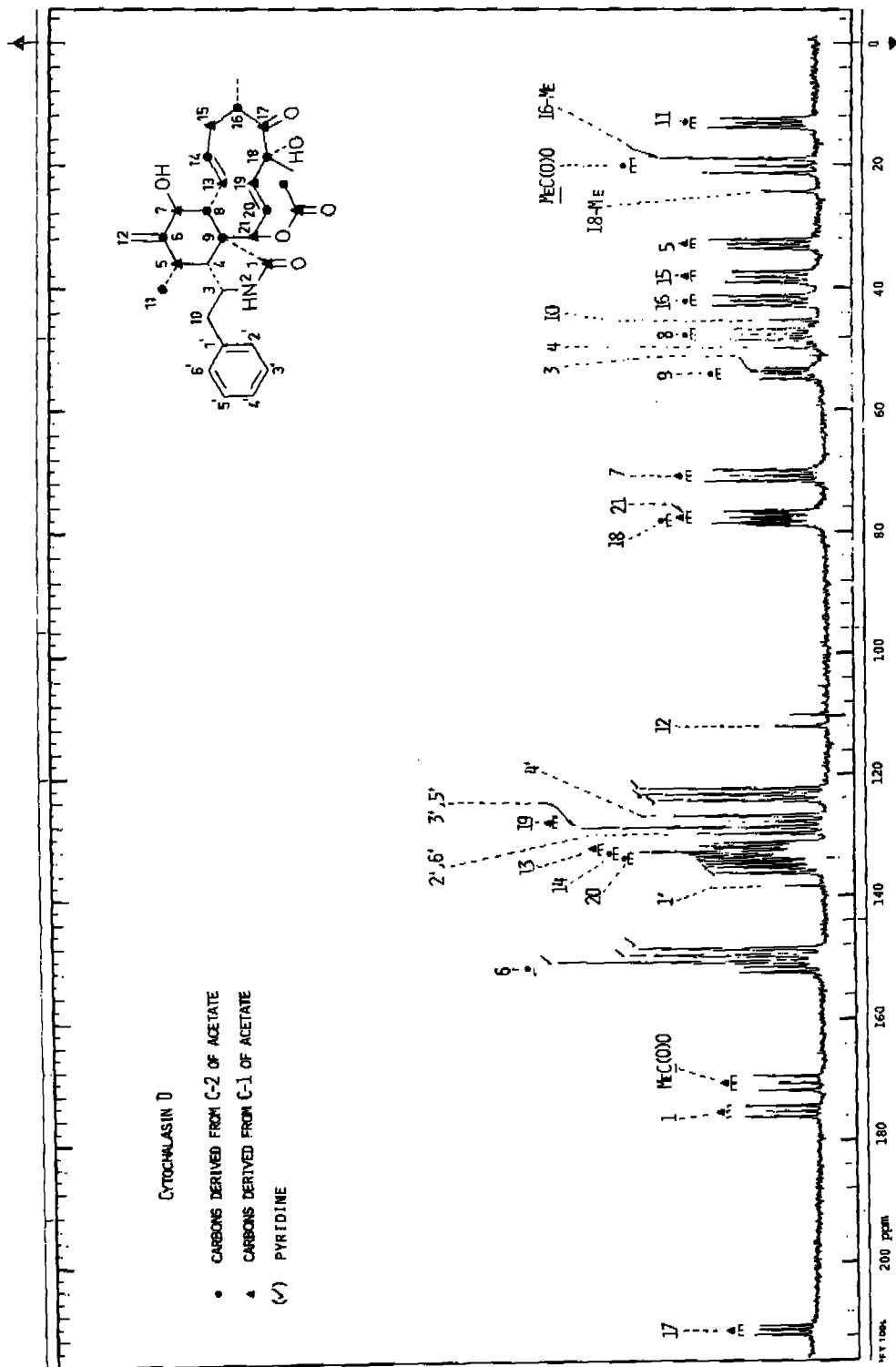


Fig. 3. Proton Decoupled ^{13}C -NMR. Spectra of Cytochalasin D after Incorporation of Sodium $[1,2,^{13}\text{C}]$ -Acetate. For experimental conditions see [1].

3. Incorporation of Other Carboxylic Acids. – After localization of the acetate units, utilization of other common acids became the focal point of investigation. A high absolute incorporation rate of 1.8% was obtained with sodium [2-¹⁴C]-propionate (Table 1), and analogous degradations showed that labelling was similar to that with sodium [1-¹⁴C]-acetate. This is in accord with the well-known biological decarboxylative fission of propionate to acetate by an α -oxidation path [25a].

Studies on the biosynthesis of cytochalasin B (2) had suggested that sodium [1-¹⁴C]-propionate was primarily incorporated in the perhydro-isoindolone moiety, in contrast to sodium [2-¹⁴C]-propionate which was distributed as [1-¹⁴C]-acetate [20]. Although the incorporation rate of sodium [1-¹⁴C]-propionate in cytochalasin D (1) was low (Table 1), cleavage of the product to the epimeric acids 17a and 17b indicated an analogously high enrichment (90%) in the lactam part. Feeding of sodium [1-¹³C]-propionate to *Z. masonii* produced a slight (10%) but probably significant enhancement at C(4) in the ¹³C-NMR. spectrum of 1 (Table 3)⁸⁾. While most of the propionate oxidatively decarboxylates to acetate with loss of radioactivity, some is transformed to phosphoenolpyruvate which condenses with shikimic acid to eventually produce C(1)-labelled phenylalanine [25b]; this yields C(4)-labelled cytochalasin D (1) [21]. Fermentation in a totally defined synthetic medium lacking phenylalanine still allowed generation of limited quantities of 1, but failed to increase the incorporation rate of sodium [1-¹⁴C]-propionate. High activity in the washed mycelium indicated that permeability problems were not serious.

Table 3. Incorporation of ¹³C-Precursors

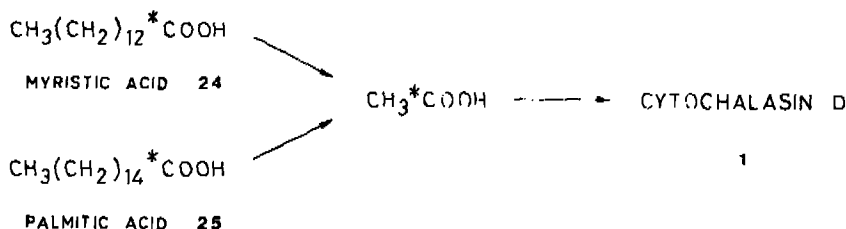
Precursor	Cytochalasin D (1)				
	Amount (mg)	Isotopic Purity %	Amount (mg)	Absolute Incorporation Rates ¹⁴ C (%)	¹³ C (%) ⁸⁾
sodium [1- ¹³ C]-acetate	530	55	97	2.4	0.86
sodium [2- ¹³ C]-acetate	519	90	131	1.2	1.5
sodium [1, 2- ¹³ C]-acetate	600	90	20	1.2–2.4	0.23
sodium [1- ¹³ C]-propionate	980	90	115	0.12	0.05
[1- ¹³ C]-myristic acid	1793	91	156	0.65	0.59
[1- ¹³ C]-palmitic acid	2865	91	199	0.08	0.47

⁸⁾ See Expcr. Part, 2. The absolute ¹³C-incorporation rates were determined by mass spectrometry [1].

Cell permeation was an obstacle in the utilization of sodium [1-¹⁴C]- and [2-¹⁴C]-malonates resp. (Table 1). Both were incorporated to a lesser extent than the corresponding acetates despite their later appearance in the biosynthetic sequence. This could be alleviated by their incorporation as diethyl esters; the conversion of diethyl [2-¹⁴C]-malonate is comparable with that of sodium [2-¹⁴C]-acetate.

An attempt to determine whether a non-branched polyketide chain **A** or branched chains **C** (Fig. 2) are responsible for the acetate-derived section of cytochalasin D (**1**) prompted additional experiments. Although the activity of washed mycelium was high, radioactive formate and [5-¹⁴C]-mevalonate gave low incorporations (Table 1). Sodium butyrate proved to be a moderately good precursor because of its facile cleavage by β -oxidation to acetate [25c].

Reports of intact incorporation of palmitic acid into brefeldin A encouraged similar studies with *Z. masonii* [26]⁹⁾. A number of trials with [1-¹⁴C]-myristic acid and [1-¹⁴C]-palmitic acid gave surprisingly high conversions (Table 1), despite apparent scrambling of label as demonstrated by drastic hydrolysis of cytochalasin D (**1**) to the acids **17a** and **17b**. Reaction of sodium [¹³C]-cyanide with 1-bromotridecane and with 1-bromopentadecane followed by basic hydrolysis produced [1-¹³C]-myristic acid (**24**) and [1-¹³C]-palmitic acid (**25**) resp. in excellent yields. Incorporation of these precursors into cytochalasin D (**1**) confirmed that degradation by β -oxidation to acetate had occurred. In agreement with results described above, and with the feeding of [1-¹³C]-acetate [1], carbon atoms C(1), C(21), C(19), C(17), C(15), C(13), C(7), C(5), and the carbonyl of the O-acetyl group of cytochalasin D (**1**) were equally enriched (about 100%) within experimental error.



To sum up, cytochalasin D (**1**) originates from phenylalanine, methionine, and nine intact acetate units. Eight of the latter couple in head to tail fashion to form the C₁₆-polyketide moiety. Utilization of common fatty acids occurs by one of two pathways. Transformation by α -oxidation accounts for distribution of C(2)-labelled propionate as C(1)-labelled acetate, and of C(1)-labelled propionate as inactive acetate and C(1)-labelled phosphoenolpyruvate. Higher even-numbered saturated acids such as C(1)-labelled butyrate, myristate, and palmitate undergo β -oxidation to yield C(1)-labelled acetate. These results and the even distribution of acetate label strongly suggest formation of a non-branched highly oxidized C₁₆-chain. Final choice between this hypothesis and one involving branched chains which are cleaved between the acetate units (**C**, Fig. 2) must await further isolation and incorporation experiments.

The support of these investigations by the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* (Project No. 2.0550.73) is gratefully acknowledged. J. C. V. thanks the *American Philosophical Society (Penrose Fund)* for a travel grant. L. D. thanks 'l'Organisation du Trait  de l'Atlantique Nord' for financial support.

Experimental Part

1. *General Methods.* Melting points were determined on a Kofler block and are corrected. Dry nitrogen was used in reactions requiring an inert atmosphere. Anhydrous sodium sulfate was employed as drying agent in reaction work-ups. Progress of most reactions and purity of all products

⁹⁾ The results have recently been disputed [27].

were checked by thin layer chromatography (TLC.) on silica gel F 254 (*E. Merck*, Darmstadt) with visualization by UV-light, iodine vapour or oxidative spray reagents. Column chromatography with *Merck* silica gel (70–230 mesh) and preparative TLC. with *Merck* silica gel PF 254 were generally used to purify products. All substances were dried at least 4 h at 0.01 Torr and 25° before spectral measurements, microanalyses or radioactivity determinations.

Microanalyses were performed in the microanalytical laboratory of the Institute (*E. Thommen*). IR., UV. and 90-MHz-NMR. spectra were measured on a *Perkin-Elmer* Model 125 grating spectrometer, a *Beckman* D.K.2 spectrophotometer and a *Bruker* WH 90 spectrometer, resp., in the spectral laboratories of the Institute (*K. Aegerter*). We gratefully acknowledge Prof. *K. Nakanishi* and Drs. *P. Solomon* and *I. Miura* (Columbia University, New York) for determination of ^{13}C -NMR. spectra which were determined on a *JEOLCO* PS-100 (25.149 MHz) instrument using partially relaxed *Fourier* transform with a $180^\circ\text{--}\tau\text{--}90^\circ\text{--T}$ pulse sequence. Additional ^{13}C -NMR. spectra were recorded on a *Bruker* WH 90 (22.63 MHz) spectrometer at the Institute laboratories in Basle (*K. Aegerter*). We thank Dr. *H. Lichti*, Sandoz AG., Basle, for all mass spectral measurements, especially in connection with determination of ^{13}C content. The spectra were measured on a *CEC* 21-110 B instrument at 70 eV. We are indebted to Mr. *H. Galliker* and Mr. *C. Marbach*, Sandoz AG., Basle, for radioactivity determinations which were carried out either directly on a *Packard* Tri-Carb Model 3375 liquid scintillation spectrometer or by prior combustion.

Radioactive precursors were purchased from *Amersham Radiochemical Centre* (Buckinghamshire, England). ^{13}C -labelled compounds were obtained from *Radium Chemie* (9053 Teufen AR, Switzerland), *Sharpe & Dohme GmbH* (Munich) and *Prochem – British Oxygen Company Ltd.* (London).

2. *Incorporation of Precursors into Cytochalasin D* (1). Unless otherwise noted, *Zygosporium masonii* was fermented for 96 h on 1 l scale as previously described [21]. A number of trials demonstrated that most precursors were advantageously added prior to inoculation of the culture medium. Incorporation of myristic acid and palmitic acid was improved by addition of these precursors in ethanol solution (5 ml) 48 h after inoculation, accompanied by enough 3N HCl to bring the medium pH to 5.0. After extraction and purification in the described fashion, cytochalasin D (1) was recrystallized to constant specific activity from acetone/hexane.

3. *Desacetylcytochalasin D* (3). Preparation of desacetylcytochalasin D (3) from cytochalasin D (1) in the usual way proceeded quantitatively to produce material with the same physical and spectral properties reported earlier [21].

4. *Kuhn-Roth Oxidation*. The following general procedure was employed for cleavage of acetic acid units out of substances 3, 7, 14, 17a, 17b, 21a and 21b. A 5.0 ml portion of *Kuhn-Roth* oxidizing solution (10 g CrO_3 in 60 ml H_2O and 15 ml conc. H_2SO_4) was mixed with 0.05 mmol of substance, sealed, and heated 2 h at 135°. The mixture was cooled and then steam distilled until 200 ml of distillate had been collected. This aqueous acetic acid solution was titrated to pH 8.5 with 0.01N NaOH and concentrated *in vacuo* to yield sodium acetate which was subjected to the *Schmidt* degradation. Titration yields of acetic acid ranged from 75% to 90%.

5. *Schmidt Degradation of Sodium Acetate*. A mixture of 0.35 mmol sodium acetate, 100 mg NaN_3 and 2.5 g polyphosphoric acid/water (95:5) was warmed to 90° during 1.5 h at 14 Torr. Evolved CO_2 was trapped in 30 ml of an aqueous solution containing 48 g $\text{Ba}(\text{OH})_2 + 18$ g BaCl_2/l . The BaCO_3 was centrifuged under N_2 , washed with water and methanol, and dried under an IR. lamp (60–90% yield). Dilution of the polyphosphoric acid solution of methylamine with water (15 ml) and basification with 30% NaOH was followed by steam distillation directly into a solution of picric acid (200 mg picric acid in 5 ml ethanol and 25 ml H_2O). Concentration of the distillate (300 ml) *in vacuo* and extraction of soluble picric acid from the residue with boiling benzene gave methylamine picrate which was recrystallized from ethanol. The pure picrate (65–80% yield) had m.p. 204–207° (dec.) and properties identical with authentic material.

6. *Dodecahydrocytochalasin D* (6). Hydrogenation of cytochalasin D (1) produced two epimeric dodecahydrocytochalasins D (6) (total yield 97%) which could be separated and had properties identical with those described previously [21]. Generally the mixture of isomers was deacetylated directly.

7. *Desacetyldodecahydrocytochalasin D* (7). Hydrolysis of the isomeric mixture of 6 generated the two corresponding epimers of 7 as described in [21].

8. *Ozonolysis of Cytochalasin D (1)*. An ozone/oxygen mixture was bubbled through a solution of 1.20 g **1** in 100 ml methanol at -75° until a deep blue color persisted. Reduction of the cold solution with 2.57 g sodium borohydride was followed by warming to 25° over 1 h, acidification with 80 ml of 2N H_2SO_4 , and concentration *in vacuo* to 60 ml. This solution was extracted with methylene chloride for 6 days in a *Kutscher-Stendel* continuous extractor. The dried extracts were concentrated *in vacuo* to give 1.31 g of colorless gum, which was directly acetylated with a mixture of 12 ml each of acetic anhydride and pyridine for 4 days at 25° . Concentration *in vacuo* yielded 1.73 g of a gummy mixture which was chromatographed on 200 g of silica gel (70–230 mesh) with methylene chloride/methanol gradient. Further purification by TLC. gave the following: 490 mg (45%) of tri-*O*-acetyl- γ -lactam **12**; 290 mg (22%) of penta-*O*-acetyl- γ -lactam **13**; 503 mg (85%) of 1,6-diacetoxy-2,4-dimethyl-2,3-diol **10**; 19 mg (3.1%) of 1,3,6-triacetoxy-2,4-dimethyl-2-ol **11**.

Tri-O-acetyl- γ -lactam 12. M.p. $77-79^{\circ}$. - IR. (KBr): 3400 (OH, NH); 2930 (CH); 1745 (C=O; acetate); 1690 (C=O; lactam); 1430, 1370, 1240, 700 cm^{-1} . - NMR. (CDCl_3): 7.26 (m, 5 H phenyl); 5.86 (s, 1H, NH, exchanged with D_2O); 5.73 (X of ABX, 1H-C(21), $J_{AX} = 5$, $J_{BX} = 3$); 5.04 (A of ABX, 1H-C(6), $J_{AB} = 6$, $J_{AX} = 6$); 4.60 (A of ABX, 1H-C(20), $J_{AB} = 13$, $J_{AX} = 3$); 4.06 (B of ABX, 1H-C(20), $J_{AB} = 13$, $J_{BX} = 5$); 4.2–3.5 (m, 4H an C(3) + C(7) + C(13)); 3.01 (A of ABX, 1H-C(10), $J_{AX} = 4$, $J_{AB} = 13$); 2.68 (B of ABX, 1H-C(10), $J_{AB} = 13$, $J_{BX} = 0$); 2.6–1.8 (m, 3H an C(4) + C(5) + C(8)); 2.16 (s, 3H, CH_3CO); 2.14 (s, 3H, CH_3CO); 2.08 (s, 3H, CH_3CO); 1.05 (d, 3H-C(11), $J = 7$). - MS.: m/e 492 ($M^+ + 1$); 491 (M^+); 474 ($M^+ - 17$, H_2O); 461; 442; 431 ($M^+ - 60$, CH_3COOH); 400 ($M^+ - 91$, benzyl); 382 ($M^+ - 109$, benzyl + H_2O); 358 ($M^+ - 133$, benzyl + acetyl); 91 (base peak).

$\text{C}_{25}\text{H}_{33}\text{NO}_9$ (491) Calc. C 61.09 H 6.77 N 2.85% Found C 60.87 H 6.82 N 2.92%

Penta-O-acetyl- γ -lactam 13. M.p. $168-171^{\circ}$. - IR. (KBr): 3360 (NH); 2980, 2950 (CH); 1740 (C=O, acetate); 1705 (C=O, lactam); 1430, 1370, 1240, 705 cm^{-1} . - NMR. (CDCl_3): 7.26 (m, 5H, phenyl); 5.79 (X of ABX, 1H-C(21), $J_{AX} = 5$, $J_{BX} = 3$); 5.65 (s, 1H, NH, exchanged with D_2O); 5.37 (A of ABX, 1H-C(6), $J_{AB} = 6$, $J_{AX} = 6$); 4.94 (B of ABX, 1H-C(7), $J_{AB} = 6$, $J_{BX} = 12$); 4.70 (A of ABX, 1H-C(20), $J_{AB} = 13$, $J_{AX} = 3$); 4.63 (A of ABX, 1H-C(13), $J_{AB} = 5.5$, $J_{AX} = 7$); 4.26 (B of ABX, 1H-C(20), $J_{AB} = 13$, $J_{BX} = 5$); 4.14 (B of ABX, 1H-C(13), $J_{AB} = 5.5$, $J_{BX} = 12$); 3.97 (X of ABX, 1H-C(3), $J_{AX} = 4$, $J_{CX} = 9$, $J_{BX} = 0$); 3.02 (A of ABX, 1H-C(10), $J_{AX} = 4$, $J_{AB} = 13$); 2.65 (B of ABX, 1H-C(10), $J_{AB} = 13$, $J_{BX} = 0$); 2.8–2.2 (m, 3H an C(4) + C(5) + C(8)); 2.17 (s, 3H, CH_3CO); 2.12 (s, 6H, $2\text{CH}_3\text{CO}$); 2.07 (s, 3H, CH_3CO); 2.03 (s, 3H, CH_3CO); 1.01 (d, 3H-C(11), $J = 7$). - MS.: m/e 576 ($M^+ + 1$); 575 (M^+); 532 ($M^+ - 43$, acetyl); 516 ($M^+ - 59$, acetoxy); 484 ($M^+ - 91$, benzyl); 442 ($M^+ - 133$, benzyl + acetyl); 424 ($M^+ - 151$, benzyl + acetic acid); 91 (base peak).

$\text{C}_{29}\text{H}_{37}\text{NO}_{11}$ (575) Calc. C 60.51 H 6.48 N 2.43% Found C 60.51 H 6.39 N 2.50%

1,6-Diacetoxy-2,4-dimethyl-hexan-2,3-diol (**10**). This mixture of two epimers possessed spectral and chromatographic properties identical with those previously reported [21].

1,3,6-Triacetoxy-2,4-dimethyl-hexan-2-ol (**11**). The two epimers had the same characteristics as those described earlier [21].

9. *Hydrolysis of Penta-O-acetyl- γ -lactam 13 to Lactam 14*. A solution of 83 mg of **13** in 100 ml methanol was stirred with 7 ml of 2N Na_2CO_3 for 7 h at 25° . Water was added, the methanol was removed *in vacuo*, and the aqueous solution was continuously extracted with methylene chloride in a *Kutscher-Stendel* apparatus for 3 days. Concentration *in vacuo* of the dried extracts and TLC. of the residue produced 23 mg (45%) of lactam **14**. - M.p. $102-104^{\circ}$. - IR. (KBr): 3350 (OH, NH); 2920 (CH); 1670 (C=O); 700 cm^{-1} . - NMR. (CD_3OD): 7.24 (m, 5H phenyl); 4.3–3.4 (m, 8H an C(3) + C(6) + C(7) + C(13) + C(20) + C(21)); 2.9–1.7 (m, 5H an C(4) + C(5) + C(8) + C(10)); 0.86 (d, 3H C(11), $J = 7$).

10. *Hydrolysis of Tri-O-acetyl- γ -lactam 12 to Lactam 14*. Treatment of **12** as described above for lactam **13** generated lactam **14** in 47% yield. Properties of this substance were identical with those of lactam **14** obtained from **13**.

11. *Hydrolytic Cleavage of Cytochalasin D (1) to Acids 17a and 17b*. A solution of 1.50 g **1** in 100 ml of methanol was treated with 2.13 g K_2CO_3 in 20 ml of water, and the mixture was refluxed 24 h under nitrogen. Dilution of the cooled solution with 70 ml of water and concentration *in vacuo* to 60 ml was followed by extraction of 792 mg of non-acidic material with methylene chloride. Acidification of the aqueous phase with 2N HCl, extraction with methylene chloride, and con-

centration of the extracts *in vacuo* produced 719 mg of a mixture of epimeric acids **17a** and **17b**. Preparative T.L.C. with 7% ethanol/methylene chloride allowed separation of 187 mg *trans*-acid **17a** and 296 mg *cis*-acid **17b** which were recrystallized from methylene chloride/hexane. Removal of solvent from the neutral fraction, consisting primarily of desacetylcytochalasin D (**3**), and further hydrolysis, as before, gave an additional 397 mg of **17a** and **17b** mixture.

Trans-acid 17a. M.p. 137–139°. – IR. (CHCl₃): 3420 (OH, NH); 2970, 2930 (CH); 1705 (C=O; acid, lactam); 970, 905 cm⁻¹. – NMR. (CDCl₃): 7.65 (m, 5H, phenyl); 6.51 (s, 1H, NH, exchanged with D₂O); 5.53 (m, 2H an C(13) + C(14)); 5.15 (s, 1H–C(12)); 4.90 (s, 1H–C(12)); 3.80 (d, 1H–C(7), *J* = 9); 3.56 (m, 1H–C(3)); 3.03–1.93 (m, 10H); 1.17 (d, 3H–C(16), methyl, *J* = 5); 1.00 (d, 3H–C(11), *J* = 7). – MS.: *m/e* 383 (*M*⁺); 365 (*M*⁺–18, H₂O); 337 (*M*⁺–46, HCOOH); 310 (base peak, *M*⁺–73, C₆H₅O₂); 274 (*M*⁺–109, benzyl + H₂O); 91.

C₂₅H₂₉NO₄ (383) Calc. C 71.13 H 7.87% Found C 71.18 H 7.91%

Cis-acid 17b. M.p. 134–137°. – IR. (CHCl₃): 3480 (OH); 3420 (NH); 2930 (CH); 1695 (C=O; acid, lactam); 980, 905 cm⁻¹. – NMR. (CDCl₃): 7.61 (s, 1H, NH, exchanged with D₂O); 7.27 (m, 5H, phenyl); 6.07 (m, 1H–C(13)); 5.63 (m, 1H–C(14)); 5.26 (s, 1H–C(12)); 5.09 (s, 1H–C(12)); 3.76 (d, 1H–C(7), *J* = 10); 3.47 (m, 1H–C(3)); 3.0–2.01 (m, 9H); 2.73 (s, 1H, OH, exchanged with D₂O); 1.77 (d, 3H–C(16), methyl, *J* = 14); 0.97 (d, 3H–C(11), *J* = 6). – MS.: *m/e* 383 (*M*⁺); 365, 354, 336, 310, 292 (*M*⁺–91, benzyl); 274, 91.

C₂₅H₂₉NO₄ (383) Calc. C 71.13 H 7.87% Found C 70.95 H 7.82%

12. Trans-lactam 21a. – An ozone/oxygen stream was bubbled through a solution of 160 mg *trans*-acid **17a** in 40 ml of methanol at –75° until a deep blue colour persisted. Sodium borohydride (1.1 g) was added portionwise to the cold solution which was then allowed to warm to 25° during 1 h. Dilution with water and removal of the methanol *in vacuo* preceded continuous extraction of the mixture with methylene chloride in a *Kutscher-Studel* apparatus for 26 h. Concentration of the dried extracts produced 94 mg of pure *trans*-lactam **21a**. – M.p. 189–192°. – IR. (KBr): 3380 (NH, OH); 2890 (CH); 1680 (C=O, lactam); 1070, 1010, 700 cm⁻¹. – NMR. ((CD₃)₂CO): 7.30 (m, 5H, phenyl); 7.07 (s, 1H, NH, exchanged with D₂O); 5.64 (m, 1H, OH, exchanged with D₂O); 3.82 (m, 1H, OH, exchanged with D₂O); 2.82 (s, 1H, OH, exchanged with D₂O); 4.1–2.2 (m, 8H); 2.0–1.5 (m, 3H an C(4) + C(5) + C(8)); 0.84 (d, 3H–C(11), *J* = 6). – MS.: *m/e* 305 (*M*⁺); 288 (*M*⁺–17, OH); 256; 214 (*M*⁺–91, benzyl); 196 (*M*⁺–109, benzyl + H₂O); 91.

C₁₇H₂₃NO₄ (305) Calc. C 66.86 H 7.59% Found C 66.65 H 7.48%

13. Cis-lactam 21b. Ozonolysis and reduction of *cis*-acid **17b** by the method described above for the corresponding *trans*-acid **17a** gave the *cis*-lactam **21b** in comparable yield. – M.p. 158–160°. – IR. (KBr): 3350 (NH, OH); 2920 (CH); 1665 (C=O; lactam); 1065, 1020, 700 cm⁻¹. – NMR. (CDCl₃): 7.29 (m, 5H phenyl); 6.11 (s, 1H NH, exchanged with D₂O); 5.46 (m, 1H OH, exchanged with D₂O); 3.96 (s, 1H OH, exchanged with D₂O); 3.34 (s, 1H, OH, exchanged with D₂O); 4.13–3.34 (m, 5H); 2.81 (m, 2H–C(10)); 2.5–1.5 (m, 4H); 0.93 (d, 3H–C(11), *J* = 7). – MS.: *m/e* 305 (*M*⁺); 288 (*M*⁺–17, OH); 275 (*M*⁺–30, CH₂O); 262 (*M*⁺–43, HNCO); 258 (*M*⁺–47, CH₂O + OH); 256; 214 (*M*⁺–91, benzyl); 196 (*M*⁺–109, benzyl + H₂O); 91.

14. Schmidt Degradation of Acids 17a and 17b. A mixture of 34.6 mg *trans*-acid **17a**, 100 mg NaN₃, and 1.9 g polyphosphoric acid/water (95/5) was warmed to 90° during 1.5 hours at 14 Torr. Evolved CO₂ was trapped in 10 ml of an aqueous solution containing 48 g Ba(OH)₂ + 18 g BaCl₂/l. The precipitate was centrifuged under nitrogen, washed with water and methanol and dried 3 h under an IR. lamp to give 3.4 mg BaCO₃ (**18**) (19% yield). *Cis*-acid **17b** was degraded by the same procedure. Results of separate radioactivity measurements of **18** obtained from *cis* and from *trans* material were within experimental error, and were therefore averaged.

15. [7-¹³C]-Myristic Acid (24). Preparation by a modification of the procedure of Anker [28]: a mixture of 2.10 g 1-bromotridecane, 40 ml of dimethylsulfoxide and 0.40 g of sodium [¹³C]-cyanide (91.5% isotopic purity) was stirred 4 days at 25°. Addition of 8.1 g sodium hydroxide in 10 ml water was followed by heating at 60° for 2 days. After removal of solvent by lyophilization, the residue was treated with 4 g of sodium hydroxide in 50 ml of water/ethanol solution (3:1) and heated under reflux for 2 days. Ethanol was distilled from the mixture, and the cooled aqueous phase was acidified with 6N HCl and extracted with methylene chloride. Concentration *in vacuo* of the dried extracts and chromatography of the residue on silica gel with hexane/ether gave 1.80 g

(98% yield) of pure [$1-^{13}\text{C}$]-myristic acid (**24**) with m.p. 52–53° and with chromatographic behaviour and [^1H]-NMR. spectra identical with those of authentic unlabelled material.

16. [$1-^{13}\text{C}$]-Palmitic Acid (**25**). Synthesis of this substance from sodium [^{13}C]-cyanide (91.5% isotopic purity) and 1-bromopentadecane by the method described above for **24** gave a 96% yield. The resulting [$1-^{13}\text{C}$]-palmitic acid (**25**) had m.p. 61–62°, and chromatographic behaviour and [^1H]-NMR. spectra identical with authentic unlabelled material.

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