Terpenoids. XIV. The Constitution and Biogenesis of Marasmic Acid[1,2]

J. J. Dugan, P. de Mayo, M. Nisbet, J. R. Robinson,[3] and M. Anchel[4]

Contribution from the Department of Chemistry, University of Western Ontario, London, Ontario, Canada. Received March 3, 1966

Abstract: Evidence is presented to demonstrate that marasmic acid has the structure 9. A reasonable biosynthetic pathway for the genesis of this compound through a humulene precursor is suggested and supported by the activity of the acetic acid obtained by the Kuhn–Roth oxidation of labeled 9 and of 15, a reduction product of marasmic acid in which a methyl group is generated from the cyclopropane ring.

Marasmic acid was first reported by Kavanagh, Hervey, and Robbins[5] during their general survey of the constituents of Basidiomycetes having antibacterial activity. This substance, isolated from *Marasmius conigenus*, showed marked activity against *Staphylococcus aureus* and slight activity against *Escherichia coli*. It was attributed the empirical formula $C_{16}H_{20}O_4$ and, on the basis of some diagnostic tests, the tentative conclusion was reached that marasmic acid was unsaturated, contained a carbonyl group, and although not phenolic, contained an acid function also.

Reculturing of the mold and growth on beech shavings in a corn steep medium has led (see the Experimental Section) to the reisolation of marasmic acid, and its identity was confirmed by comparison with an original specimen. Repetition of the analysis gave figures in better agreement with the formula $C_{15}H_{18}O_4$, and the molecular weight (262) required by this formula was confirmed by a mass spectrometric determination.

Of the four oxygen atoms in marasmic acid one could be attributed to the carbonyl group of an α,β-unsaturated carbonyl system (λ_{max} 241 mμ (ϵ 9700) and ν_{max} 1684 and 1631 cm^{-1}). Two more were probably combined in a γ-lactone (ν_{max} 1773 cm^{-1}), while the fourth was present as an hydroxyl group (ν_{max} 3350 cm^{-1}; deuterium exchangeable proton in its nmr spectrum). The nmr spectrum further indicated that the carbonyl function was an aldehyde (singlet proton at τ 0.57) in which the α carbon was fully substituted and that at the β position bore one proton (τ 3.50) split by at least one adjacent proton (at the γ position).

Methylation of marasmic acid with diazomethane left the unsaturated aldehyde function untouched. However, the absorption in the infrared spectrum indicative of the presence of the γ-lactone and of the hydroxyl group was now replaced by a band at 1724 cm^{-1} suggesting the formation of a carboxylic ester. These observations were compatible with the transformation of part-structure **1** → **2**. The remaining details indicated in the part-structures were required by the observation of a signal at τ 3.87 (for the proton attached

to the lactol ether terminus) and of the additional aldehydic signal at τ 0.17, and by the fact that both of these signals were singlets.
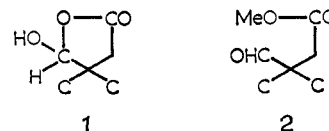
In addition to the carbon atoms included in **1**, and those required by the presence of the α,β-unsaturated aldehyde, two further carbon atoms could be identified. These were recognized as being methyl groups attached to quaternary carbon by the absorption at τ 8.96 and 8.93 and the nmr spectrum. Finally, from the empirical formula and the functional groups noted it could be concluded that, since marasmic acid contained no further point of unsaturation, it was tricarbocyclic.

Information about the carbon skeleton present in marasmic acid was obtained as follows. Reduction with sodium borohydride converted the lactol to a lactone and reduced the aldehyde to an allylic alcohol. The product, on heating with palladized charcoal,[6] gave a mixture, the hydrocarbon portion of which was examined by gas–liquid partition chromatography. It consisted largely of two aromatic compounds which were readily recognized from their spectra as methylated indanes.

The first of these compounds (A) was found to be a C_{13} methylated indane. The presence of two aromatic methyl groups was indicated by the two three-hydrogen singlets at τ 7.92 and 7.82. A six-hydrogen singlet at τ 8.87 revealed the presence of two *equivalent* quaternary methyl groups. These and other details of the nmr spectrum required that the unsymmetrical indane be represented by **3** or **4**. A standard synthesis of **3** from *m*-xylene by acylation with β-chloropropionyl chloride, alkylation, and reduction gave a substance different from A, from which it was concluded, albeit on the basis of negative evidence, that A was to be represented by **4**.

The second hydrocarbon (B) was a methylated indane containing 14 carbon atoms. The nmr spectrum showed that B contained three aromatic methyl groups. Bearing in mind the structure of A, B then had to be represented by **5** or **6**. Syntheses of **5** and **6** from 1,2,3-

(1) Part of this work was reported in preliminary form: J. J. Dugan, P. de Mayo, M. Nisbet, and M. Anchel, *J. Am. Chem. Soc.*, 87, 2768 (1965).
(2) This research was supported, in part, by the National Research Council of Canada.
(3) Canada Department of Agriculture, University of Western Ontario, London, Canada.
(4) New York Botanical Garden, New York, N. Y.
(5) F. Kavanagh, A. Hervey, and W. J. Robbins, *Proc. Natl. Acad. Sci. U. S.*, 35, 343 (1949).

(6) The process cannot be described as a dehydrogenation since after the nature of the transformations involved had been ascertained it appeared that the over-all change was one of simple dehydration and bond cleavage.

and 1,2,4-trimethylbenzene, respectively, gave hydro-carbons, the first of which was found to be identical with B. In the latter synthesis the two indanones produced in the acylation were separated and one (7) was methylated and reduced.

3   4

5   6   7
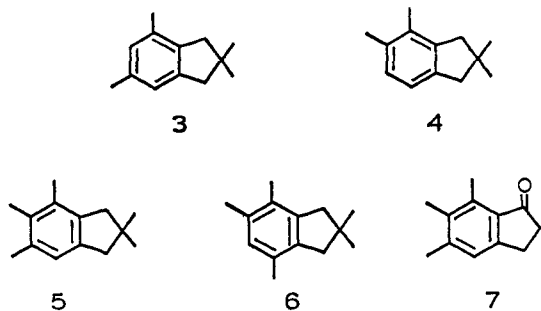
The indane **5** contained 14 of the 15 carbon atoms of marasmic acid, and it could be assumed that the atom lost was that of the lactol carbonyl (as carbon dioxide). Furthermore, this same indane contained two of the three carbocyclic rings required by the final structure. The following facts further limited the possibilities available.

First, the carboxyl carbon had to be γ to the saturated aldehyde so as to permit lactol formation. Second, since the proton attached to the lactol ether terminus was a singlet (as was the saturated aldehydic proton in marasmic ester) one of the termini of the third ring had to be at this point. On the assumption for the moment, because of the ready conversion to indanes, that the remaining ring was three membered,[7] the number of structural representations possible for marasmic acid could be reduced to three (**8–10**).


8   9   10
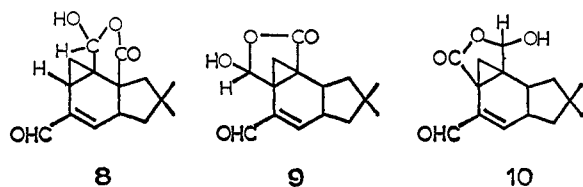
In all these structures there was present a 1,2 or 1,3 relationship between the two aldehydes. There is much evidence that such an arrangement greatly facilitates a Cannizzaro reaction.[8] Treatment of marasmic acid with 5% aqueous alkali under reflux gave, after methylation with diazomethane, a crystalline product. This substance had the spectral properties expected for an α,β-unsaturated ester (λ_{max} 233 mμ; ν_{max} 1709 cm^{-1}) and for a γ-lactone (ν_{max} 1767 cm^{-1}). There was also a proton at the β position in the unsaturated ester function.

These properties required that the products to be expected from the three possible structures for marasmic acid be represented as **11–13**, respectively.[9]

(7) Evidence for the presence of a cyclopropyl ring in marasmic acid was also available in the nmr spectrum of methyl marasmate. An AB pattern centered at τ 8.26 (J_{AB} = 5 cps, $\delta_B - \delta_A$ = 70.8 cps) could be discerned, but at the early stages of the investigation the appearance of this band at low field raised some doubt as to its exact significance.

(8) For an example in a 1,2-dialdehyde see C. S. Barnes and J. W. Loder, *Australian J. Chem.*, **15**, 322 (1962). For 1,3-dialdehydic cases see P. de Mayo and A. N. Starratt, *Can. J. Chem.*, **40**, 1632 (1962); G. W. K. Cavill, D. L. Ford, and H. D. Locksley, *Australian J. Chem.*, **9**, 288 (1956).

(9) In the cases of **8** and **10**, migration of the ethylenic linkage to the β,γ position is required prior to the occurrence of the Cannizzaro reaction.

11   12   13
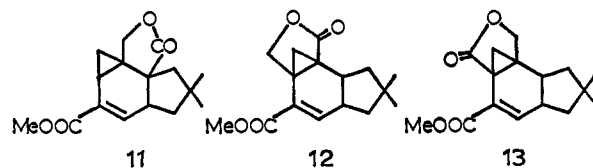
Hydrogenation of this new ester, with interruption after the uptake of 1 molecular equiv of hydrogen, gave a substance which, like its precursor, was a γ-lactone and contained a conjugated ester. It differed in two important respects. First, the substance now possessed *three* quaternary C-methyl groups. The presence of the third group was indicated by the appearance of a three-hydrogen singlet at τ 8.71 and an increase in the Kuhn–Roth value from 0.45 to 1.37. The only interpretation possible for this observation is that of the cleavage, by hydrogenolysis, of a cyclopropane ring.

Secondly, the dihydro compound while still, as stated, an α,β-unsaturated ester had not retained the ethylenic linkage in the original position: no vinyl proton was now to be discernible in the nmr spectrum. This, in conjunction with the first observation, suggested that a 1,4 over-all addition of hydrogen had occurred. On this basis **11** could be discarded as a representation of the Cannizzaro compound since 1,4 addition would have given a substance (**14**) still retaining a vinyl proton; **15** and **16** remained acceptable at this point, for substances derived from **12** and **13**, respectively. A distinction between these possibilities could be made as follows. As required by **12** or **13** the presence of a methylene group attached to oxygen could be observed in the nmr spectrum of the Cannizzaro ester (AB pattern centered at τ 5.52). In the dihydro compound this signal had moved downfield to τ 4.83 indicating the proximity of an ethylenic linkage. This was clearly true of **15**, but not of **16**. On hydro-

14   15   16

17
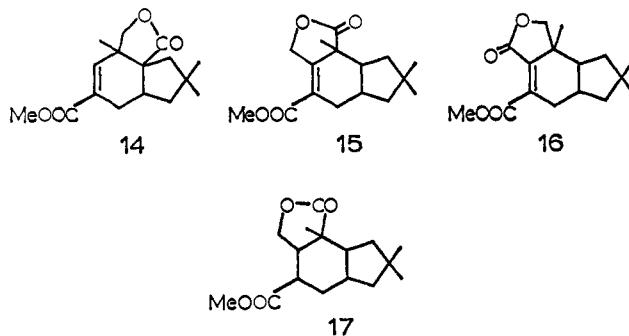
genation of **15** a further molecular equivalent of hydrogen was taken up. The resultant product (**17**), a saturated ester,[10] had absorption, attributed to the methylene group of the γ-lactone, at τ 5.77. This represents an upfield shift of 0.94 ppm, acceptable for the saturation of an "allylic" double bond.

On the evidence so far accumulated **9** could be tentatively accepted as denoting the structure of marasmic acid, but more evidence for the interrelationship of the functional groups was evidently desirable.

It was clear that **12** contained an array of functions arranged in such a manner that generation of the carbanion from the ester would be expected to induce a reverse Michael reaction with cleavage of the cyclo-

(10) The ester carbonyl had ν_{max} 1734 cm^{-1} confirming that in the precursors the conjugation was to be attributed to an α,β-unsaturated ester function and not an α,β-unsaturated lactone.

propane ring.[11] Indeed treatment of **12** with 5% methanolic sodium methoxide at reflux temperature for 30 min led to the generation of a substance with the empirical formula $C_{16}H_{20}O_4$, having an extended chromophore in the ultraviolet: this absorption (λ_{max} 274 mμ) was compatible with that to be expected for the cross-conjugated system indicated in **18**.[13]

The dienic system bore but one proton (indicated by a broad singlet at τ 3.86). The ester function remained and was unconjugated (ν_{max} 1736 cm^{-1}). The remaining two oxygen atoms were present in γ-lactone, probably α,β-unsaturated (ν_{max} 1754 cm^{-1}). A methylene group attached to oxygen was indicated by a broad, two-proton singlet at τ 5.30, and two quaternary methyl groups by singlets at 9.03 and 8.90. These results are easily accommodated in the representation **18** formed by a reverse Michael reaction in the methanolyzed lactone **19** to give **20**, followed by relactonization with the second carbomethoxyl group.
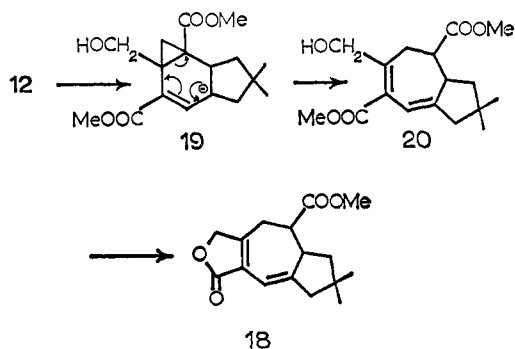
Attempts were also made to induce the opening of the cyclopropane ring in marasmic acid itself. With acetic acid–hydrochloric acid at 110° marasmic acid was converted into a mixture from which was isolated a chlorine-containing substance to which the structure **21** is presently attributed. This interpretation is based on the following evidence.

The ultraviolet spectrum showed a maximum at 215 mμ (ϵ 5300) compatible with the presence of a furan ring.[14] The presence of two vinylic protons on carbon bearing oxygen was indicated by two one-proton bands at τ 2.76 and 2.66. The absence of other vinyl protons in the spectrum required substitution at the β positions of the furan. The chloromethyl protons occurred as an AB pattern centered at τ 6.12 and the lack of further splitting indicated attachment to a quaternary carbon

(11) The situation is a vinylogous extention of that found in homocaronic ester i which has been shown to be converted to ii under the conditions of basic hydrolysis.[12]
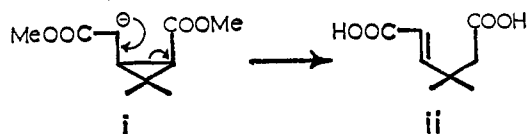
(12) G. Widmark, *Arkiv Kemi*, **11**, 195 (1957).
(13) The less substituted cyclohexyl derivative (iii) is reported to have λ_{max} 264 mμ: E. A. Braude and E. A. Evans, *J. Chem. Soc.*, 607 (1954).
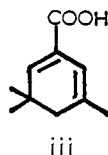

(14) See A. I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Pergamon Press Ltd., London, 1964, p 137.

atom. Two three-proton singlets at τ 8.92 and 8.80 implied the continuing presence of the two methyl groups in the cyclopentane ring. The ethereal oxygen of a γ-lactone (ν_{max} 1776 cm^{-1}) was attached to quaternary carbon also, since there was no signals to be attributed to protons on carbon bearing oxygen.

The remaining seven protons could also be identified. The allylic methylene group formed an AB pattern, with fine splitting because of coupling with the furan protons, centered at τ 7.08,[15] while the methylene attached α to the lactonic oxygen formed an AB pattern centered at τ 7.30. The AB portion had the A and B patterns centered at τ 8.15 and 8.65, respectively.[15]

In agreement with the formulation of this substance as a furan a Diels–Alder adduct was obtained with acetylene dicarboxylic ester. All the observed spectral data were in accord with expectations for **22**, but, in particular, the furanic proton signal in **21** had been replaced by signals at τ 4.45 and 4.20 for the same protons now on saturated carbon bearing oxygen.
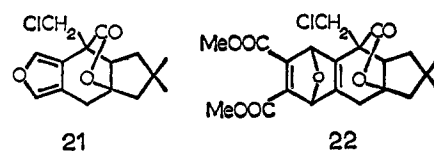
The formation of **21** in itself, with the presence of a chloromethyl group, supported the view that a cyclopropane ring was present in marasmic acid. The formation of a furan is classical evidence for the presence of a 1,2-dialdehyde in the molecule. But since the over-all transformation was one of some complexity, and the reagent one of demonstrated vigor,[16] the efficaciousness of milder conditions was investigated though without success.

The use of deuterium chloride (instead of hydrogen chloride) in acetic acid-d_1 resulted in the formation of **21** which contained three deuterons. Two of these were those included in the furan moiety.[18] The third was such as to provide some insight into the model of genesis of **21**.

One of a number of ways in which the formation of the furan might be thought to occur is indicated in the sequence **23** → **24**. The final lactonization, sterically
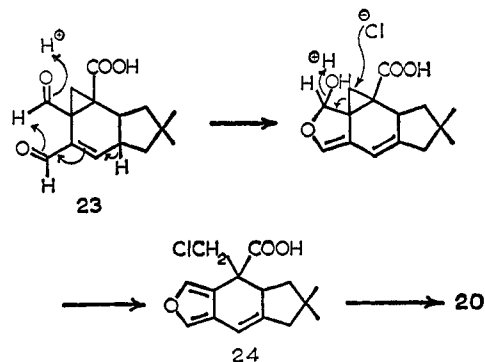
possible only β to the furan, requires protonation α to the furan. Under deuterating conditions, then, a

(15) The details of the spectrum are recorded in the Experimental Section.
(16) As for instance in the conversion of friedelene to olean-13,8-ene.[17]
(17) G. Brownlie, F. S. Spring, R. Stevenson, and W. S. Strachan, *J. Chem. Soc.*, 2419 (1956).
(18) These were probably incorporated by exchange in the furan itself.

deuteron should have been introduced at that point. It was found that the AB pattern centered at τ 7.08 which had been assigned to this group had been replaced by a one-proton broad singlet at τ 6.85. On irradiation of the deuterium[19] at its resonance frequency, this band collapsed to a sharp singlet. It thus appeared that the deuterium been incorporated at this point and stereospecifically so.

A final attempt was made to achieve the cleavage of the cyclopropane ring in marasmic by a process other than brutal. It was considered that the chloro compound **25** might solvolyze under appropriate conditions with concomitant ring cleavage. The preparation of **25** was attempted by allowing marasmic acid to stand in dimethylformamide containing thionyl chloride at 0°. The infrared spectrum showed the presence of the α,β-unsaturated aldehyde, but, in addition, a band at 1751 cm^{-1} indicated the existence of a saturated aldehyde. A further band (at 1792 cm^{-1}) suggested the presence of an acid chloride. The compound was accordingly assigned the structure **26**.

At room temperature, in contrast, a substance was obtained exhibiting only two carbonyl bands, at 1802 and 1686 cm^{-1}, in the infrared spectrum. Only one aldehydic proton signal, at τ 0.50, was evident, and in this substance the cyclopropane methylene group was more clearly evident than in others of the series. It appeared as an AB pattern centered at τ 8.59 (J_{AB} = 5 cps, $\delta_B - \delta_A$ = 18.7 cps). The formation of this substance, clearly the desired **25**, from **26** is reminiscent of the dual behavior of succinyl chloride and related substances. Attempted solvolysis of **25** at 140° in dimethylformamide containing lithium chloride led to no cyclopropane cleavage, and the only product, other than starting material, obtained was marasmic acid.

25   26
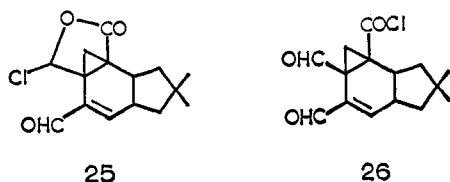
Aside from the stereochemistry of the lactol hydroxyl group, marasmic acid contains four asymmetric centers. Since there had to be a *cis* fusion between the cyclopropane ring and the cyclohexene ring, there were two relationships to be determined to define the relative stereochemistry of the marasmic acid carbon skeleton. These were the hydrindane ring fusion, and the relationship of the lactonic carbonyl to this junction.

From an inspection of models it appeared that in substance **21** the chloromethyl group and the adjacent proton had to be in a *cis* relationship, the alternative structure being very highly strained.[20] This steric relationship could not be transferred to marasmic acid without demonstration that this proton was not inverted during the genesis of the furan. That no inversion had taken place was a permissible conclusion was indicated by the experiment wherein the furan (**21**)

was prepared under deuterating conditions, but in which no incorporation occurred at this point.

Evidence was available from two experiments to indicate that the hydrindane ring fusion in marasmic acid was in the more stable configuration. Treatment of marasmic acid with isopropenyl acetate containing p-toluenesulfonic acid gave an amorphous enol acetate (**27**; λ_{max} 248 mμ) in which the lactol hydroxyl group was also acetylated. With aqueous acetic acid-hydrochloric acid in the cold, marasmic acid was regenerated. Further, when marasmic acid was heated under reflux in deuterium oxide containing p-toluenesulfonic acid, 0.6 deuteron was incorporated and marasmic acid was recovered. The location of the deuteron was indicated by the collapsing of the signal attributed to the vinyl proton.

Regrettably, however, it remains unclear from the inspection of models whether the *cis*- or *trans*-fused isomers should be the more stable, and therefore this point of stereochemistry, from the present work, must remain unsettled.

The structure represented by **9** while evidently that of a terpenoid is not to be derived by simple farnesol cyclization. A likely biogenetic route to its formation appeared to be that through an ion such as **28** which could arise through a humulene-type precursor (**29**). Migration of bond a in **28** would lead to **32** which has the carbon skeleton of marasmic acid, while migration of bond **b** produces the gross structure of the illudins as found, for instance, in illudin-S[21-23] (**31**) and, in fact, is the route suggested for the probable genesis of these substance by their investigators.[21,22]

To test this hypothetical scheme, marasmic acid was grown on slopes in a medium containing ($\pm$)-mevalonic acid-2-[14]C. After dilution the material was crystallized to constant activity, the incorporation being near 0.3%.

On the basis of the suggested scheme all activity should reside in the atoms indicated in **33**. Insufficient material was available for systematic degradation, and so recourse was had to Kuhn-Roth oxidation. Such oxidation of marasmic acid would result in the formation of acetic acid from the geminal dimethyl group, while oxidation of the ester **15** would, in addition, produce acetic acid from the carbon atom originally constituting the cyclopropylmethylene group. On the assumption that the yield of acetic acid from the geminal dimethyl group remained constant the yield of acid from the new methyl group could be calculated. In both cases the carbon atom adjacent to the labeled atom was necessarily included, and so the site of the label in the second case has some ambiguity.

Estimation of the activity of the derived acetic acid (see Table I and the Experimental Section) and correction for yield, determined in separate experiments, and for the fact that only half the acetic acid obtained from the geminal dimethyl group was active, gave the following results.

(19) We are very much indebted to Dr. J. B. Stothers for this experiment.

(20) The argument though persuasive must be admitted not to be decisive, since the lactone was not prepared under demonstrably equilibrating conditions. However, the formation of a system with two *trans*-fused five-membered rings is very improbable.

(21) T. C. McMorris and M. Anchel, *J. Am. Chem. Soc.*, **85**, 831 (1963); **87**, 1594 (1965).

(22) T. Tada, Y. Yamada, N. S. Bhacca, K. Nakanishi, and M. Ohashi, *Chem. Pharm. Bull.* (Tokyo), **7**, 853 (1964); K. Kakanishi, M. Tada, and Y. Yamada, *ibid.*, **7**, 856 (1964); K. Nakanishi, M. Ohashi, M. Tada, and Y. Yamada, *Tetrahedron*, **21**, 1231 (1965).

(23) T. Matsumoto, H. Shiratama, A. Ichihara, Y. Fukuoka, Y. Takahashi, Y. Mori, and M. Watanabe, *ibid.*, **21**, 2671 (1965).

Table I. Kuhn-Roth Oxidations and Activity Determinations

Run[a]	Wt, mg	Activity, μcurie/mg	Titration, ml	Total activity μcurie/mg	Portion of CMe isolated	% of total activity of group(s)[c,d]
1	23.2	1.89×10^{-3}	4.36	3.54×10^{-3}	0.483	33.4
2	26.9	9.85×10^{-4}	4.66	1.99×10^{-3}	0.445	33.8
3	4.67	9.94×10^{-4}	2.37	1.34×10^{-3}	1.37	56.9[b]
4	4.82	9.94×10^{-4}	2.42	1.54×10^{-3}	1.36	61.2[b]

[a] Runs 1 and 2 in marasmic acid, 3 and 4 in dihydro Cannizzaro ester (15). [b] Assuming that the amount of acetic acid derived from the geminal group is the same as in marasmic acid. [c] Corrected for partial oxidation in Kuhn-Roth oxidation. [d] All radioactivity measurements were made by chromic acid oxidation of the sample, followed by assay of $C^{14}O_2$ using an ionization chamber and vibrating-reed electrometer.

Approximately one-third of the total activity in the molecule resided in the geminal dimethyl group while about 26% resided in the cyclopropane methylene group (or in the adjacent carbon atom). While these results are not sufficiently definitive to compel the acceptance of the suggested biogenetic scheme, it is
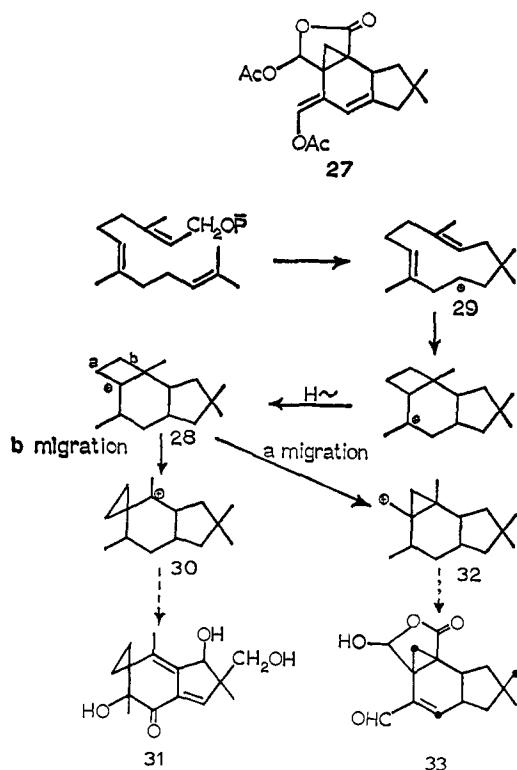
considered that they are adequate to transpose it from the area of the intrinsically plausible to that of the temperately probable.

Experimental Section[24]

Surface Culturing of *Marasmius conigenus.* A layer of beech shavings was placed in the bottom of a Rouse bottle or a 4-qt milk bottle, and medium (55 and 120 ml, respectively) was added. The medium was prepared by dissolving corn steep solids[25] (5 g), dextrose (40 g), sodium nitrate (3 g), potassium dihydrogen phosphate (1 g), potassium chloride (0.5 g), and magnesium sulfate heptahydrate (0.5 g) in 1 l. of distilled water. The bottles were sterilized by autoclaving at 15 psi for 25 min and inoculated with *M. conigenus.* A complete mat of mold covered the Beech support after 2–3 months of incubation at 29°. The medium was poured

(24) Melting points were determined on a Kofler hot stage unless otherwise stated. Ultraviolet spectra are in methanol solution. The nmr spectra were recorded on a Varian A-60 spectrometer and are reported in τ units.
(25) The corn steep solids were in Special Nutrient No. 14 from the A. E. Staley Co.

off under sterile conditions and fresh sterilized medium (75 and 150 ml, respectively) was added. This process was repeated every 7 days until the mat became contaminated. Most slopes were changed regularly for a period of up to 6 months at which time the yield of marasmic acid began to decline sharply.

Isolation of Marasmic Acid. A total of 5 l. of medium from 35 Rouse and 13 milk bottles was divided into two lots. Each was filtered through cotton into a 4-l. separatory funnel and extracted four times with ether (500 ml each time). The combined ether extract was evaporated to dryness at 40°. The product was dissolved in 100 ml of chloroform and extracted twice into 5% sodium carbonate (80 ml each time). The carbonate solution was acidified to pH ~2 with hydrochloric acid and extracted three times with chloroform. Evaporation to dryness at 40° gave 1275 mg of crude marasmic acid.

The crude acid (2.05 g) was chromatographed on silica gel (66 g) using ether–benzene mixtures as eluent. The fractions found to contain marasmic acid (by tlc (eluent, ether)) were crystallized from ethyl acetate and gave nearly pure marasmic acid (800 mg). Additional crystallizations from ethyl acetate gave analytically pure material; mp 173–174° (evacuated capillary); $[\alpha]_D +182°$ (c 1.38); λ_{max} 241 mμ (ϵ 9700); ν_{max} 3350, 1773, 1684, and 1631 cm^{-1}; nmr bands at τ 8.93 and 8.96 (6 H, two singlet), 8.59 (2 H, AB pattern, $J_{AB} = 5$ cps, $\delta_B - \delta_A = 16.8$ cps), 3.87 (1 H, singlet), 3.50 (1 H, doublet, $J \sim 2$ cps), and 0.57 (1 H, singlet).

Anal. Calcd for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.38; H, 6.60.

Methyl Marasmate. Marasmic acid (100 mg) was methylated with a slight excess of diazomethane. Separation of the product by tlc (eluent, ether) gave homogeneous material (98 mg) which was crystallized from petroleum ether (bp 60–80°)–ethyl acetate. The compound had mp 89.5–91.0°, $[\alpha]_D +126°$ (c 1.81), λ_{max} 243 mμ (ϵ 10,200); ν_{max} 1724, 1672, and 1637 cm^{-1}; nmr bands at τ 8.95 (6 H, singlet), 8.26 (2 H, AB pattern, $J_{AB} = 5$ cps, $\delta_B - \delta_A = 70.8$ cps), 6.34 (3 H, singlet), 3.45 (1 H, doublet, $J \sim 2$ cps), 0.52 (1 H, singlet), and 0.17 (1 H, singlet).

Anal. Calcd for $C_{16}H_{20}O_4$: C, 69.54; H, 7.30. Found: C, 69.89; H, 6.97.

Sodium Borohydride Reduction of Marasmic Acid. Marasmic acid (107 mg) was dissolved in methanol (10 ml), and sodium borohydride (157 mg) was added slowly while the solution was being cooled. The solution was allowed to stand for 45 min, acidified to pH ~2, and extracted into chloroform. The product was heated on a steam bath overnight under vacuum. Separation by tlc (eluent, ether) and distillation at 120° and 0.001 mm gave an alcohol, $[\alpha]_D +36°$ (c 1.93), λ_{max} 203 mμ (ϵ 6400), ν_{max} 1765 cm^{-1}; nmr bands at τ 8.98 and 9.87 (6 H, two singlets), 4.77 (2 H, broad singlet), 5.57 (2 H, singlet), and 4.72 (1 H, broad singlet).

Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.01; H, 7.78.

Dehydrogenation. The sodium borohydride reduction product (750 mg) was dehydrogenated by heating with palladized charcoal (5% 1 g) at 300° for 2 hr under a slow stream of nitrogen. The charcoal was washed with acetone and ether to give a brown oil (195 mg) which was distilled at 100° (0.3 mm) to give a clear oil (120 mg). Vpc on a 3% DEGS (diethylene glycol succinate) column at 125° showed two major products (A and B).

2,2,4,5-Tetramethylindane (Compound A). A (26 mg), isolated by preparative vpc, had λ_{max} 269 mμ (ϵ 1200), 273 (1150), and 278 (1150); nmr bands at τ 8.87 (6 H, singlet), 7.92 (3 H, singlet), 7.82 (3 H, singlet), 7.38 (4 H, broad singlet), and 3.25 (2 H, broad band) in CCl$_4$.

Anal. Calcd for $C_{11}H_{18}$: C, 89.59; H, 10.41. Found: C, 89.19; H, 10.58.

2,2,4,5,6-Pentamethylindane (Compound B). B (31 mg), also isolated by preparative vpc, had λ_{max} 271 mμ (ϵ 1100), 276 (960), and 281 (1120); nmr bands at τ 8.87 (6 H, singlet), 7.92 (6 H, singlet), 7.82 (3 H, singlet), 7.40 (4 H, broad singlet), and 3.33 (1 H, broad singlet) in CCl$_4$.

Synthesis of 2,2,4,5,6-Pentamethylindane. A. A solution of 1,2,3-trimethylbenzene (30 g) and chloropropionyl chloride (39.4 g) in carbon disulfide (30 ml) was added dropwise over a period of 1 hr to a stirred solution of aluminum chloride (50 g) in carbon disulfide (200 ml). Stirring was continued for 2.5 hr and the carbon disulfide evaporated under vacuum at room temperature. Concentrated sulfuric acid (300 ml) was added slowly and the mixture heated at 80–90° for 2 hr and then set aside for 36 hr at room temperature. The solution was poured onto crushed ice, and extraction into ether gave a brown oil (27.9 g). Tlc (eluent, ether–benzene, 1:3) indicated two products and a preparative separation of 390 mg gave two compounds. The first (250 g) was distilled at 80° (0.001 mm) to give a clear oil which was crystallized from methanol to give 5,6,7-trimethylindan-1-one. It had mp 55–56°, ν_{max} 1695 cm^{-1}, λ_{max} 259 mμ (ϵ 14,100) and 305 mμ (ϵ 2900); nmr bands at τ 7.82 (3 H, singlet), 7.66 (3 H, singlet), 7.38 (3 H, singlet), 7.58–7.28 (2 H, complex band), 7.20–6.92 (2 H, complex band), and 2.91 (1 H, singlet).

Anal. Calcd for C$_{12}$H$_{14}$O: C, 82.72; H, 8.10. Found: C, 82.81; H, 7.64.

The second (90 mg) compound was distilled at 80° (0.001 mm) to give an oil which on crystallization from methanol yielded 4,5,6-trimethylindan-1-one: mp 77–78°, ν_{max} 1695 cm^{-1}, λ_{max} 263 mμ (ϵ 13,000) and 304 mμ (ϵ 3200); nmr bands at τ 7.74 (6 H, singlet), 7.70 (3 H, singlet), 7.57–7.26 (2 H, complex multiplet), 7.20–6.91 (2 H, complex band), and 2.56 (1 H, singlet).

Anal. Calcd for C$_{12}$H$_{14}$O: C, 82.72; H, 8.10. Found: C, 83.10; H, 7.98.

B. 5,6,7-Trimethylindanone (500 mg) and methyl iodide (1.5 ml) were added to a solution of potassium t-butoxide (from 540 mg of potassium) and the solution was refluxed under nitrogen for 3.5 hr. The product (500 mg) was separated by tlc (eluent, ether–benzene, 1:9) followed by distillation at 80° (0.001 mm) and gave pure 2,2,5,6,7-pentamethylindan-1-one: ν_{max} 1695 cm^{-1}, λ_{max} 260 mμ (ϵ 16,700) and 306 mμ (ϵ 3500); nmr bands at τ 8.82 (6 H, singlet), 7.82 (3 H, singlet), 7.66 (3 H, singlet), 7.36 (3 H, singlet), 7.17 (2 H, singlet), and 2.94 (1 H, singlet).

Anal. Calcd for C$_{14}$H$_{18}$O: C, 83.12; H, 8.97. Found: C, 82.92; H, 8.95.

The 2,4-dinitrophenylhydrazone, recrystallized from chloroform–methanol, had mp 252–253° (evacuated capillary): ν_{max} 3330, 1613, and 1590 cm^{-1}; λ_{max} 386 mμ (ϵ 29,600).

Anal. Calcd for C$_{20}$H$_{22}$N$_4$O$_4$: C, 62.81; H, 5.80; N, 14.65. Found: C, 63.01; H, 5.71; N, 14.48.

C. Triethylene glycol (30 ml) containing the ketone (308 mg), hydrazine hydrochloride (5.05 g), and hydrazine (20 ml, 95+%) was refluxed for 14 hr at an inner temperature of 135°. Potassium hydroxide (6 g) was added slowly and volatile material boiled off until the solution refluxed smoothly at 220°. The mixture was refluxed at this temperature, under nitrogen, for 3 hr. Isolation of the product and distillation at 100° (0.2 mm) gave a clear oil (190 mg). Preparative vpc separation on a DEGS (3%) column at 130° gave the required hydrocarbon, λ_{max} 271 mμ (ϵ 1050), 276 (920), and 281 (1110); nmr bands at τ 8.87 (6 H, singlet), 7.92 (6 H, singlet), 7.82 (3 H, singlet), 7.40 (4 H, broad singlet), and 3.33 (1 H, broad band) in CCl$_4$.

Anal. Calcd for C$_{14}$H$_{20}$: C, 89.29; H, 10.71. Found: C, 89.09; H, 10.42.

The infrared spectrum of this compound was identical with that of compound B obtained in the dehydrogenation.

Synthesis of 2,2,5,7-Tetramethylindane. A. A solution of m-xylene (21.2 g) and chloropropionyl chloride (26.6 g) in carbon disulfide (25 ml) was added dropwise over a period of 45 min to a stirred mixture of aluminum chloride (32 g) in carbon disulfide (125 ml). After 3 hr at room temperature, the carbon disulfide was distilled off leaving a brown oil. Concentrated sulfuric acid (200 ml) was carefully added and the mixture was heated at 90° for 45 min. After standing overnight at room temperature, crushed ice was added and the mixture was extracted with ether. Crystallization of the product (16.1 g) from methanol gave the indanone: mp 76–77°; ν_{max} 1695 cm^{-1}; λ_{max} 256 mμ (ϵ 10,000) and 294 mμ (ϵ 1800).

B. The ketone (6 g) in t-butyl alcohol (50 ml) was added to a solution of potassium (4.68 g) in dry t-butyl alcohol (200 ml). Methyl iodide (15.5 ml) was cautiously added and the mixture was

refluxed for 2.5 hr. Distillation of the product at 100° (0.3 mm) gave 2,2,5,7-tetramethylindan-1-one (4.6 g): λ_{max} 260 mμ (ϵ 14,100) and 292 mμ (ϵ 2200); nmr bands at τ 8.87 (6 H, singlet), 7.67 (3 H, singlet), 7.47 (3 H, singlet), 7.18 (2 H, singlet), 3.18 (1 H, broad singlet), and 3.10 (1 H, broad singlet).

Anal. Calcd for C$_{13}$H$_{16}$O: C, 82.93; H, 8.57. Found: C, 82.53; H, 8.20.

The 2,4-dinitrophenylhydrazone on crystallization from chloroform–methanol had mp 222–224° (evacuated capillary); ν_{max} 3300, 1613, and 1590 cm^{-1}; λ_{max} 384 mμ (ϵ 30,500).

Anal. Calcd for C$_{19}$H$_{20}$N$_4$O$_4$: C, 61.94; H, 5.47; N, 15.21. Found: C, 61.94; H, 5.42; N, 15.87.

C. The ketone (360 mg), potassium hydroxide (0.2 g), and hydrazine (0.3 ml, 95+%) in triethylene glycol (4 ml) were refluxed for 2 hr at 190°. Low-boiling material was distilled and the temperature was raised to 210°. The mixture was maintained at this temperature for 3 hr. The product, after distillation at 80° (0.2 mm), gave an oil (160 mg). Preparative vpc separation on a 3% Dow 710 column gave the hydrocarbon, 2,2,5,7-tetramethylindane (50 mg), and starting material (76 mg). The hydrocarbon had λ_{max} 265 mμ (ϵ 640), 269 (820), and 279 (845); nmr bands at τ 8.88 (6 H, singlet), 7.88 (3 H, singlet), 7.78 (3 H, singlet E, 7.45 (2 H, broad singlet), 7.38 (2 H, broad singlet), and 3.36 (2 H, broad band) in CCl$_4$.

Anal. Calcd for C$_{13}$H$_{18}$: C, 89.59; H, 10.41. Found: C, 89.86; H, 10.41.

Synthesis of 2,2,4,5,7-Trimethylindane. A. A solution of 1,2,4-trimethylbenzene (24 g) and chloropropionyl chloride (26.6 g) in carbon disulfide (25 ml) was added over a period of 45 min to aluminum chloride (32 g) in carbon disulfide (125 ml). The reaction was carried further as previously to yield a solid (17 g) from methanol. Recrystallization from methanol gave 4,6,7-trimethyl-indan-1-one, mp 110–111°, λ_{max} 262 mμ (ϵ 10,700) and 304 mμ (ϵ 1700).

Anal. Calcd for C$_{12}$H$_{14}$O: C, 82.72; H, 8.10. Found: C, 83.05; H, 8.11.

B. The ketone (5 g) was methylated as previously to give a solid product from methanol (4.2 g). Recrystallization gave 2,2,4,6,7-pentamethylindan-1-one: mp 75–76°; λ_{max} 262 mμ (ϵ 13,000) and 304 mμ (ϵ 1900); nmr bands at τ 8.87 (6 H, singlet), 7.88 (3 H, singlet), 7.75 (3 H, singlet), 7.52 (3 H, singlet), 7.25 (2 H, broad singlet), and 3.20 (1 H, broad singlet).

Anal. Calcd for C$_{14}$H$_{18}$O: C, 83.12; H, 8.97. Found: C, 83.27; H, 8.58.

C. The ketone (210 mg), hydrazine hydrate (3.99 ml, 85%), and hydrazine dihydrochloride (830 mg) in triethylene glycol (5 ml) were refluxed at an inner temperature at 130° for 2.5 hr. Potassium hydroxide (2.4 g) was added and the temperature was raised slowly to 210° by distilling off low-boiling material. The temperature was maintained at 210° for 4 hr. Work-up in the usual way gave an oil (120 mg) which was separated by vpc on a 3% Dow 710 column to give the hydrocarbon (76 mg) and starting material (26 mg). The hydrocarbon had λ_{max} 270 mμ (ϵ 700); nmr bands at τ 8.87 (6 H, singlet), 7.95, 7.91, and 7.85 (9 H, three singlets), 7.40 (4 H, broad singlet), and 3.38 (1 H, broad singlet) in CCl$_4$.

Anal. Calcd for C$_{19}$H$_{20}$: C, 82.93; H, 8.57. Found: C, 82.53; H, 8.20.

Cannizzaro Ester (12). An aqueous solution of sodium hydroxide (15 ml, 5%) was degassed at reflux temperature by bubbling in nitrogen for 30 min. Marasmic acid (192 mg) was added and the solution was refluxed under nitrogen for 80 min. The solution was cooled, acidified, and extracted with chloroform. The product was methylated with diazomethane, distilled at 100° (0.2 mm), and crystallized from aqueous ethanol to give the ester (50 mg). Further crystallization from aqueous ethanol gave material: mp 127–128°; $[\alpha]_D$ +80° (c 1.38); λ_{max} 233 mμ (ϵ 6300); ν_{max} 1767, 1709, and 1634 cm^{-1}; nmr bands at τ 8.98 (3 H, singlet), 8.95 (3 H, singlet), 6.20 (3 H, singlet), 5.52 (2 H, AB pattern, J_{AB} = 10 cps, δ_B − δ_A = 20.7 cps), and 3.35 (1 H, doublet, $J \sim$ 2 cps).

Anal. Calcd for C$_{16}$H$_{20}$O$_4$: C, 69.54; H, 7.30. Found: C, 69.83; H, 7.70.

Dihydro Cannizzaro Ester (15). The Cannizzaro ester (28.3 mg) was hydrogenated in ethyl acetate (5 ml) containing 26 mg of palladized charcoal (5%). A break in the uptake of hydrogen was observed after consumption of 1 molecular equiv. The product was purified by tlc (eluent, ether–benzene, 1:7) and by crystallization from petroleum ether (bp 35–60°) to give the dihydro derivative: mp 110–111°, $[\alpha]_D$ +96° (c 1.33); λ_{max} 231 mμ (ϵ 10,100), ν_{max} 1773, 1709, and 1675 cm^{-1}; nmr bands at τ 9.03 (6 H, singlet), 8.71 (3 H, singlet), 6.23 (3 H, singlet), and 4.83 (2 H, broad singlet).

Anal. Calcd for $C_{16}H_{22}O_4$: C, 69.04; H, 7.97. Found: C, 68.76; H, 7.71.

Tetrahydro Cannizzaro Ester (17). The dihydro Cannizzaro ester (40 mg) was hydrogenated in ethyl acetate (5 ml) containing palladized charcoal (30 ml, 5%). Uptake of hydrogen stopped after the consumption of 1 molecular equiv. The product, purified by tlc (eluent, ether-benzene, 1:7) and by crystallization from petroleum ether (bp 35-60°) gave **28**: mp 93-95°; [α]D +66° (c 1.30); λmax 200 mμ (ε 380); νmax 1767 and 1734 cm⁻¹; nmr bands at τ 9.03 (3 H, singlet), 8.90 (3 H, singlet), 8.65 (3 H, singlet), 6.30 (3 H, singlet), and 5.77 (2 H, complex multiplet).

Anal. Calcd for $C_{16}H_{24}O_4$: C, 68.54; H, 8.63. Found: C, 68.76; H, 8.21.

Cleavage of the Cannizzaro Ester. The Cannizzaro ester (75.4 mg) was refluxed in dry methanolic sodium methoxide (4 ml, 5%) under nitrogen for 30 min. The reaction mixture was acidified and extracted with chloroform, and the crude product was separated by tlc (eluent, ether-benzene, 1:2) to give a product (32 mg) which, after crystallization from petroleum ether, gave **18**: mp 142-143°; [α]D +216° (c 1.11); λmax 274 mμ (ε 7500); νmax 1754, 1736, and 1686 cm⁻¹; nmr bands at τ 9.03 (3 H, singlet), 8.90 (3 H, singlet), 6.27 (3 H, singlet), 5.30 (2 H, broad singlet), and 3.86 (1 H, broad singlet).

Anal. Calcd for $C_{16}H_{20}O_4$: C, 69.54; H, 7.30. Found: C, 69.77; H, 7.96.

Marasmic Acid Enol Diacetate (27). Marasmic acid (52 mg) was refluxed in isopropenyl acetate (5 ml) containing p-toluenesulfonic acid (65 mg) for 5 hr. Separation of the product by tlc (eluent, ether-benzene, 1:9) gave the acetate as a gum (36 g); [α]D +204° (c 1.36); λmax 248 mμ (ε 19,000); νmax 1789, 1761, and 1645 cm⁻¹. This compound had nmr bands at τ 8.93 (3 H, singlet), 8.85 (3 H, singlet), 7.99 (3 H, singlet), 7.85 (3 H, singlet), 4.30 (1 H, broad band), 3.15 (1 H, singlet), and 2.73 (1 H, broad singlet).

Anal. Calcd for $C_{19}H_{22}O_6$: C, 65.88; H, 6.40. Found: C, 65.75; H, 5.96.

The enol diacetate (20 mg) was hydrolyzed by allowing it to stand in acetic acid (2.4 ml), water (1.5 ml), and hydrochloric acid (0.35 ml, concentrated) at room temperature for 24 hr. Dilution with water, extraction into chloroform, separation tlc (eluent, ether-benzene, 3:1), and crystallization from aqueous acetone gave marasmic acid (6 mg). Identity was established by melting point, mixture melting point, infrared spectra, and movement on tlc.

Treatment of Marasmic Acid with Hydrochloric Acid (Furan 20). Marasmic acid (170 mg) was heated to 110° in acetic acid (15 ml), and hydrochloric acid (0.75 ml, concentrated) was added. Heating was continued for 7 min and the solution was cooled, poured into water, and neutralized to pH ∼3. Extraction into chloroform and separation by tlc (eluent, ether-benzene, 1:9) gave a homogeneous product (40 mg). Distillation at 95° (0.001 mm) gave a clear, colorless oil (29 mg) which crystallized from petroleum ether and had mp 93-94°, [α]D −13° (c 0.97), and νmax 1776 cm⁻¹.

Anal. Calcd for $C_{15}H_{17}O_4Cl$: C, 64.30; H, 6.12; Cl, 12.65. Found: C, 64.49; H, 5.64; Cl, 12.51.

This compound had nmr bands at τ 8.92 (3 H, singlet), 8.80 (3 H, singlet), 8.04 (3 H, AB pattern, J_{AB} = 15 cps, $δ_B − δ_A$ = 26.0 cps), 7.08 (2 H, AB pattern, J_{AB} = 17 cps, $δ_B − δ_A$ = 23.5 cps), 6.12 (2 H, AB pattern, J_{AB} = 12 cps, $δ_B − δ_A$ = 21.9 cps), 2.76 (1 H, sharp band with fine splitting), 2.66 (1 H, sharp band with fine splitting), and 7.30 (1 H, doublet of doublets, X band of ABX pattern), AB of ABX (J_{AB} = 16.7 cps, J_{AX} = 8.1 cps, J_{BX} = 10.9 cps) with the A and B patterns being centered at τ 8.15 and 8.65, respectively. The deuterated product was prepared in the following manner.

Marasmic acid (200 mg) was heated to 100° in acetic acid-d (30 ml), and deuterium chloride (3 ml, 12 N) was added. The solution was kept at this temperature for 7 min. Work-up as before, followed by several recrystallizations from petroleum ether, gave furan-d_3 (15 mg), mp 93.5-94.5°, mmp 93.0-94.0°.

Anal. Calcd for $C_{15}H_{14}O_4ClD_3$: 17.6% excess deuterium. Found: 16.2% excess deuterium.

Diels-Alder Adduct (21). The furan (39 mg) was heated with acetylene dimethylcarboxylate (194 mg) in chloroform (3 ml) in a sealed tube at 105° for 12 hr. Separation by tlc (eluent, ether-benzene, 3:7) gave the adduct (42 mg) as an oil: νmax 1773, 1706, and 1638 cm⁻¹; λmax 204 mμ (ε 8700) and 225 (shoulder) mμ (ε 6800); nmr bands at 8.75 (3 H, singlet), 8.67 (3 H, singlet, 6.18 (6 H, singlet), 6.11 (2 H, AB pattern, J_{AB} = 12 cps, $δ_B − δ_A$ = 27.5 cps), 4.45 (1 H, doublet, J ∼ 1.5 cps), and 4.20 (1 H, narrow multiplet).

Anal. Calcd for $C_{21}H_{28}O_8Cl$: C, 57.45; H, 5.28. Found: C, 57.85; H, 5.95.

Treatment of Marasmic Acid with Thionyl Chloride. Marasmic acid (90 mg) was allowed to stand in dimethylformamide (8 ml) containing thionyl chloride (1.5 ml) at room temperature for 30 min. The product was poured into water and extracted into chloroform. Separation by tlc (eluent, ether-benzene, 1:3) gave a homogeneous product (**25**, 20 mg) which crystallized from petroleum ether-ethyl acetate. This compound had mp 146-150°; [α]D +244° (c 0.78); λmax 239 mμ (ε 10,200); νmax 1802, 1686, and 1637 cm⁻¹; nmr bands at τ 8.92 (6 H, singlet), 8.59 (2 H, AB pattern, J_{AB} = 5 cps, $δ_B − δ_A$ = 18.7 cps), 3.43 (1 H, doublet, J ∼ 2.5 cps), 3.33 (1 H, singlet), and 0.50 (1 H, singlet).

Anal. Calcd for $C_{15}H_{17}O_3Cl$: C, 64.15; H, 6.10; Cl, 12.63. Found: C, 64.07; H, 6.13; Cl, 12.76.

Marasmic Acid-d. Marasmic acid (200 mg) was refluxed in dioxanedeuterium oxide (20 ml, 1:1) containing p-toluenesulfonic acid (193 mg) for 10 hr. Dilution with water, and extraction into ether gave crude product. Purification by tlc (eluent, ether) gave homogeneous material (120 mg) which on crystallization from ethyl acetate had mp 174-175° (evacuated capillary). On admixture with authentic marasmic acid the melting point was 173-174° (evacuated capillary).

Anal. Calcd for $C_{15}H_{17}O_4D$: excess 5.55% deuterium. Found: 3.10% excess deuterium.

Labeled Marasmic Acid. To each of two mature slopes of *M. conigenus* in 4-qt milk bottles was added medium (150 ml) containing 25 μcuries of dl-mevalonic acid-2-C¹⁴ (salt of N,N′-dibenzylethylenediamine).[26] After 4 days of incubation at 29° the slopes were reflooded with fresh medium (150 ml). This medium was poured off after another 4 days and combined with the first batch. The combined medium was extracted four times with ether and the ether was evaporated at 40°. The extract was dissolved in chloroform which was then extracted with potassium carbonate (5%) to give crude marasmic acid (89 mg). The carbonate-soluble material was separated by tlc (eluent, ether) to give homogeneous material (53 mg).

This procedure was repeated using another total of 50 μcuries of dl-mevalonic acid-2-C¹⁴ to give 59 mg of marasmic acid.

The marasmic acid from these two experiments was combined and diluted with inactive marasmic acid to give a total of 216 mg, which was crystallized from ethyl acetate to constant specific activity (154 mg, 1.89 ± 0.02 × 10⁻³ μcurie/mg)

Labeled Dihydro Cannizzaro Ester. Marasmic acid (91 mg, 1.89 × 10⁻² μcurie mg) was converted into **12** (21 mg). This was diluted with inactive material to give 33.1 mg which was hydrogenated; 19 mg of the dihydro compound (9.94 ± 0.06 μcuries/mg) was thus obtained.

Kuhn-Roth Oxidations. A typical run was as follows. Marasmic acid (23.2 mg, 1.89 × 10⁻³ μcurie/mg) was put into the oxidation flask, and 5 ml of the cooled oxidation mixture (20 ml of 5 N chromic and 5 ml of sulfuric acid, specific gravity 1.84) was added. The mixture was refluxed for 14 hr and cooled, and dilute hydrazine solution (40%) was added dropwise, with cooling, until the first tinge of green was observed. The mixture was neutralized with sodium hydroxide (6 ml, 5 N) and then acidified with phosphoric acid (1 ml, 85%), and the acetic acid was obtained by distillation.

(26) Supplies by New England Nuclear Corp., Boston, Mass