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Sesquiterpene Biosynthesis: The Biosynthesis of Quadrone and Terrecyclic Acid

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Feeding of [1-^{13}C]- and [1,2-$^{13}C_2$]acetates to cultures of *Aspergillus terreus* gave labeled quadrone (**1**) and terrecyclic acid (**2**) which were analyzed by high-field ^{13}C NMR. The patterns of enhancements and couplings were used not only in the analysis of the biosynthetic origins of the two metabolites, but also in the assignment of the ^{13}C spectra themselves. The latter assignments were confirmed and further extended by extensive analysis by a combination of ^{1}H COSY, ^{1}H^{13}C heteroCOSY, and difference NOE spectra of quadrone. The biosynthetic pathway was further probed by incorporation of [3,4-$^{13}C_2$]mevalonate, revealing that formation of **1** and **2** involves cleavage of the 3,4-bond of one of the three mevalonte precursors. The results are consistent with the formation of quadrone and terrecyclic acid by cyclization of farnesyl pyrophosphate (**8**).

Quadrone (**1**) is a metabolite of *Aspergillus terreus* first isolated in 1978 and shown to have modest antitumor activity (*1, 2*) (Fig. 1). The challenge presented by the unusual tetracyclic lactone skeleton has prompted numerous synthetic approaches, resulting in more than a half-dozen successful syntheses by 1985 (*3–12*). The first of these syntheses, reported by Danishefsky *et al.* (*3*), involved as the last step the pyrolysis of the enone acid **2** to afford quadrone. It was therefore of some interest when the same substance **2**, dubbed terrecyclic acid, was subsequently isolated from culture filtrates of *A. terreus*, raising the possibility that this cometabolite is in fact a biosynthetic precursor of quadrone (*13, 14*). Further investigations by the groups of Hirota and Isogai have led to the isolation of additional cometabolites, including terrecyclol (**3**), (*15*), isoquadrone (**4**) (also prepared previously by Danishefsky), 8-hydroxyquadrone (**5**), and 6-hydroxyisoquadrone (**6**) (*16*). In the meantime, Smith and Konopelski (*11*) have carried out a total synthesis of (+)-quadrone, which proved to be opposite in sign of rotation to the naturally occurring levorotatory isomer, thereby establishing the absolute configuration of (−)-(**1**), a result independently confirmed by Kon *et al.* (*17*) who converted (+)-fenchone to (−)-terrecyclic acid, the enantiomer of the naturally occurring material. More recently, the latter assignment has been confirmed by CD exciton chirality methods (*18*).

Our own interest in the biosynthesis of dimethylcyclopentane sesquiterpenes (*19–23*) has led us to consider the problem of quadrone and terrecyclic acid biosynthesis. The novel tricyclic carbon skeleton of this family of fungal metabolites suggested that these compounds might represent yet another variation in the

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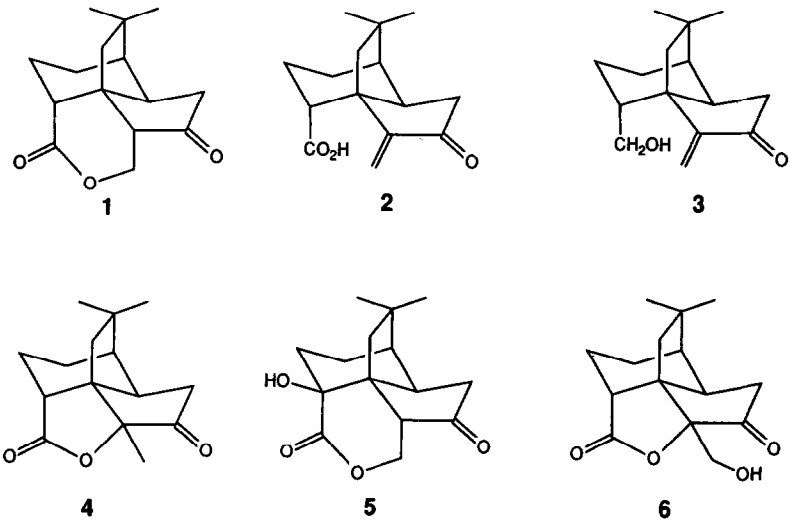

FIG. 1. Structures of quadrone (**1**), terrecyclic acid (**2**), and related metabolites of *A. terreus*.

rich spectrum of polyquinane sesquiterpenes isolated from microbial and plant sources and formally derivable by cyclization of humulene (*24*). We therefore undertook a series of incorporation experiments, designed to establish the mevalonoid origin of **1** and **2** and to elucidate the details of the key cyclization reaction by which these unusual metabolites are formed (*25*).

RESULTS

Following preliminary experiments to establish suitable conditions for quadrone and terrecyclic acid production as well as appropriate protocols for incorporation of labeled acetate, cultures of *A. terreus* were supplemented with three equal portions of sodium [1-^{13}C]acetate at intervals of 24 h beginning 74 h after initial inoculation. The incubation was carried out for a total of 172 h, after which the filtered culture broths were extracted with $CHCl_3$. The concentrated $CHCl_3$ extracts were subjected to chromatographic purification and the recovered samples of labeled quadrone and terrecyclic acid were analyzed by 62.9 MHz ^{13}C NMR. The ^{13}C-NMR spectrum of each labeled metabolite displayed, as expected, six signals which were enhanced with respect to the corresponding natural abundance spectra (Table 1 and Fig. 2). Following identical procedures, a sample of sodium [1,2-$^{13}C_2$]acetate (99 at.% ^{13}C), diluted with three parts of unlabeled sodium acetate, was administered to fermentations of *A. terreus* at 74, 98, and 122 h. The ^{13}C NMR spectra of the resulting labeled quadrone and terrecyclic acid each revealed six sets of enhanced and coupled doublets as well as three enhanced singlets, in accord with the expected isoprenoid origin of these metabolites.

Assignment of the ^{13}C-NMR spectra of **1** and **2** was based on standard chemical shift rules and off-resonance multiplicities in combination with an analysis of the

TABLE 1

^{13}C-NMR SPECTRA OF **1** AND **2** AND INCORPORATION OF LABELED ACETATES

	Quadrone[a]			Terrecyclic acid[b]		
C	δ_C(m)	[1-^{13}C] Acetate[c]	[1,2-$^{13}C_2$] Acetate[d] J_{CC}, Hz	δ_C(m)	[1-^{13}C] Acetate[c]	[1,2-$^{13}C_2$] Acetate[d] J_{CC}, Hz
2	52.6(d)		35.8	46.5(d)		35.1
3	43.2(t)	*	35.6	41.5(t)	*	35.0
4	216.2(s)		*	207.1(s)		*
5	52.3(d)	*	35.5	150.7(s)	*	72.4
6	65.3(t)		35.5	115.8(t)		72.5
7	173.8(s)		50.8	179.7(s)		56.4
8	46.1(d)	*	51.0	48.0(d)	*	55.5
9	19.4(t)		*	22.6(t)		*
10	28.9(t)	*	32.3	29.0(t)	*	32.4
11	49.0(d)		32.1	49.1(d)		32.5
1	50.0(s)		34.2	55.0(s)		32.5
12	52.8(t)	*	34.4	54.3(t)	*	32.4
13	40.5(s)	*	36.8	40.5(s)	*	36.7
14	26.9(q)		36.7	27.4(q)		36.4
15	34.9(q)		*	34.8(q)		*

* Sites of ^{13}C enrichment.

[a] $CDCL_3$, spectral width 16,129 Hz, 32 K data points, 22.5° pulse, acquisition time 1.02 s.

[b] Spectral width 15,151 Hz, 32 K data points, 12.9° pulse, acquisition time 1.08 s.

[c] A total of 0.99 g of sodium [1-^{13}C]acetate (90 at.% ^{13}C) was administered in three equal portions at 74, 98, and 122 h, respectively, to 10 100-ml cultures; avg enrichment, 3%.

[d] A mixture of 0.30 g of sodium [1,2-$^{13}C_2$]acetate (99 at.% ^{13}C) and 0.90 g of unlabeled sodium acetate was administered in three equal portions at 74, 98, and 122 h, respectively, to 11 100-ml cultures; avg enrichment, 3%.

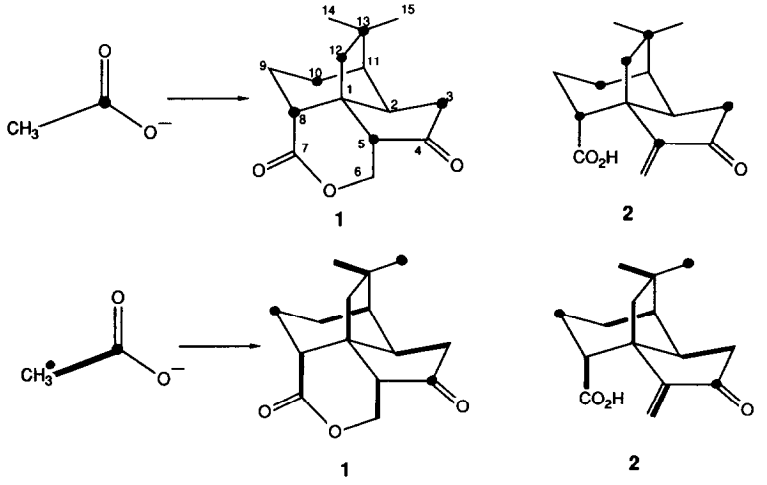

FIG. 2. Labeling of quadrone (**1**) and terrecyclic acid (**2**) by [1-^{13}C]acetate and [1,2-$^{13}C_2$]acetate.

observed labeling patterns in each metabolite. The J_{CC} coupling constants measured in the spectra of quadrone and terrecyclic acid derived from [1,2-$^{13}C_2$]acetate yielded connectivity information which provided the key to the assignment of individual resonances. Both quadrone and terrecyclic have four end groups, corresponding to C-6, -7, -14, and -15, each of which is at the terminus of a carbon chain and which therefore has a unique neighboring carbon atom. Moreover, these end groups are among the easiest to identify based on their characteristic chemical shifts. Thus the signal at 173.8 ppm in the spectrum of quadrone was readily assigned to the lactonic carbonyl, C-7. In the spectrum of quadrone derived from [1,2-$^{13}C_2$]acetate this signal was coupled (J_{CC} 50.8 Hz) to an upfield resonance at 46.1 ppm, which appeared as a doublet in the corresponding off-resonance spectrum. The latter signal was therefore uniquely assignable to the methine carbon, C-8. Similarly the lactonic methylenoxy, C-6, which appeared as the furthest downfield triplet in the off-resonance spectrum, was coupled to its unique neighbor, C-5, at 52.3 ppm (d), an assignment corroborated by $^1H^{13}C$-heteroCOSY analysis, as described below. Of the two methyl resonances, that at 26.9 ppm was coupled to its neighbor, C-13, which can be assigned to the quaternary carbon signal at 40.5 ppm on the basis of J_{CC} coupling constant (36.8 Hz). Moreover, since the paired methyl signal was not enhanced by [1-^{13}C]acetate, C-13, derived from the same unit of acetate, must necessarily have been derived from C-1 of acetate. In fact, of the two quaternary carbon resonances, only that at 40.5 ppm was enhanced by [1-^{13}C]acetate. By extension, the remaining quaternary resonance at 50.0 ppm in the spectrum of quadrone derived from [1,2-$^{13}C_2$]acetate could only be assigned to C-1, a signal which was coupled (J_{CC} 34.2 Hz) to that at 52.8 ppm, which in turn could only be the methylene carbon, C-12. Completely analogous arguments could also be used to assign the C-5,6, C-7,8, C-13,14, and C-1,12 pairs in terrecyclic acid. The carbonyl carbon, C-4, readily identifiable by its characteristic chemical shift, appeared as an enhanced singlet in the spectra of both quadrone and terrecyclic acid derived from [1,2-$^{13}C_2$]acetate. Based on the magnitude of the observed coupling constants, the two remaining methine carbons, corresponding to C-2 and C-11, could not be coupled to each other, and must therefore have been coupled to their respective neighbors, C-3 and C-10. This left only C-9, at 19.4 and 22.6 ppm in **1** and **2**, respectively, as the unique assignment for the enhanced methylene singlets.

Further confirmation of the above signal assignments was achieved by analysis of a combination of ^{1}H-COSY, resolution-enhanced one-dimensional ^{1}H-NMR, difference NOE, and heteroCOSY spectra of quadrone. Thus, H-5, which appeared as a 5.2 Hz doublet at δ 2.42, was readily identified by its characteristic cross-peak with H-6b in the ^{1}H-COSY spectrum. In the corresponding heteroCOSY spectrum, H-5 was clearly correlated to the ^{13}C resonance at 52.3 ppm, previously attributed to C-5 on the basis of J_{CC} values. H-3a and H-3b, which comprised the downfield portion of an ABX system, were correlated with the ^{13}C resonance at 43.2 ppm, while the COSY spectrum displayed a strong cross-peak with H-2 at 1.82, which in turn was correlated with the ^{13}C resonance at 52.6 ppm. In like manner, the H-8 doublet, J = 7.0 Hz, at δ 2.75 (correlated with C-8 at 46.1 ppm) showed cross-peaks with H-9a and H-9b at 1.8 and 2.35, respectively, the latter itself coupled to an H-10 signal at 1.65. Recognition of the H-9 and H-10

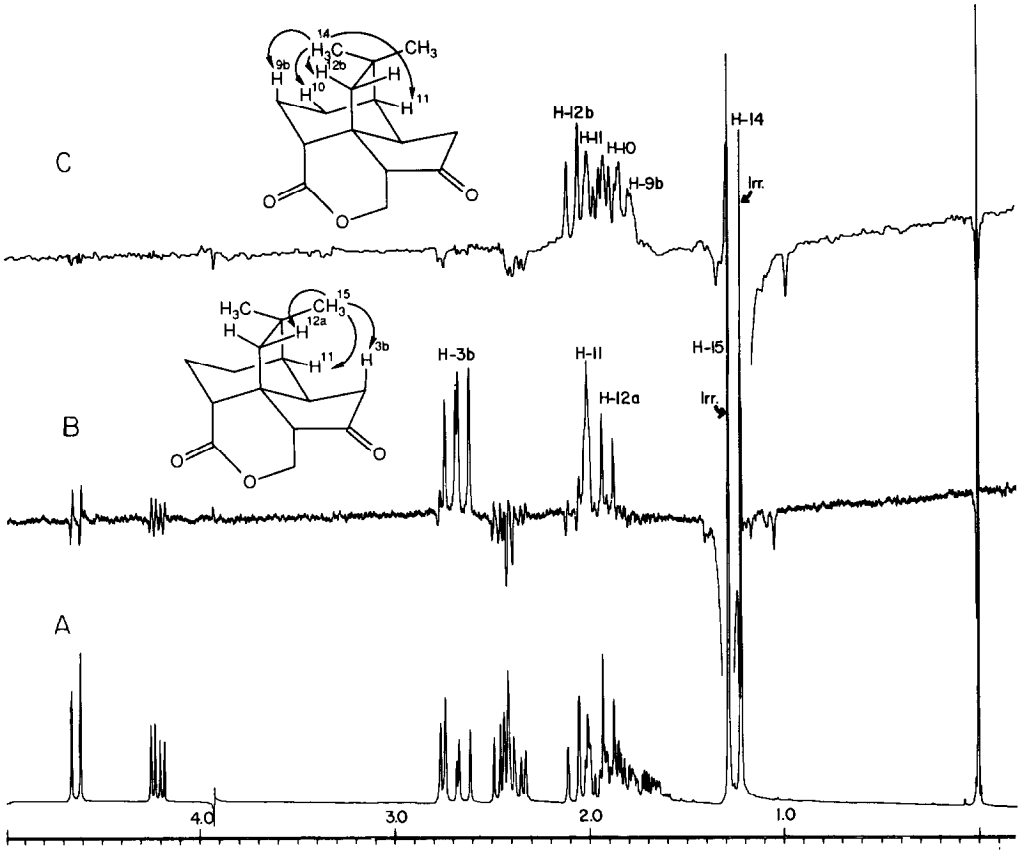

FIG. 3. Difference NOE spectra of quadrone. Spectra were accumulated in separate blocks with the decoupler alternately gated on resonance for the desired peak for four scans and 5.4 ppm upfield of TMS for four scans. Typical preirradiation times were 4× acquisition time or 10–12 s, with the decoupler being turned off during acquisition. A 3- to 10-s delay was used between irradiation modes. Difference spectra were obtained by application of a 1- to 2-Hz line broadening to each FID, followed by subtraction and Fourier transformation. (A) Quadrone, control spectrum. (B) Difference spectrum, irradiation of H-15 at 1.28 ppm. (C) Difference spectrum, irradiation of H-14 at 1.22 ppm.

signals allowed confirmation from the heteroCOSY spectrum of the previously deduced C-9 and C-10 assignments. Difference NOE analysis provided the critical stereochemical assignment of the geminal methyl resonances corresponding to H-14 and H-15 (Fig. 3). Thus irradiation of the upfield singlet at δ 1.22 gave rise to Nuclear Overhauser enhancements of the signals assigned to H-9b, H-10, H-11, and H-12b, while complementary irradiation of the H-15 methyl resonance at δ 1.28 resulted in enhancements of H-3b, H-11, and H-12a. The corresponding ^{13}C-NMR assignments followed unambiguously from the heteroCOSY spectrum, based on the observed correlation of H-14 with the upfield ^{13}C resonance at 26.9 ppm and H-15 with the downfield signal at 34.9 ppm[1] (Fig. 4).

[1] The ^{13}C-NMR assignments for terrecyclic acid are in agreement with those reported independently by Hirota *et al.* (*26*).

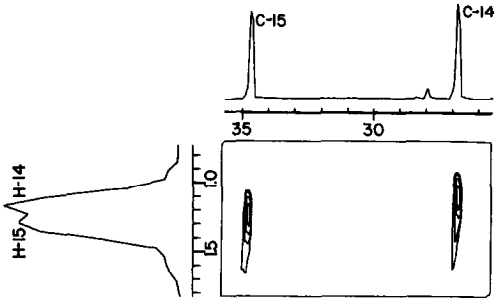

FIG. 4. Portion of $^{1}H^{13}C$-heteroCOSY spectrum showing correlation of H-14,15 and C-14,15 signals. The spectrum was acquired using a time domain of 128 in the f_1 (^{1}H) dimension (SW = 1000 Hz, 256 data points) and a time domain of 1 K in the f_2 (^{13}C) dimension (SW = 2994 Hz, 2 K data points). The ^{1}H and ^{13}C offsets were set so that the olefinic signals in each domain were folded over.

The labeling patterns observed for the incorporation of [1-^{13}C]acetate and [1,2-$^{13}C_2$]acetate into both quadrone and terrecyclic acid are typical of isopenoid metabolites (*27–29*). Thus condensation of three units of acetate is known to give mevalonic acid (**7**) which in turn is converted by well-established steps to the universal precursor of sesquiterpenes, farnesyl pyrophosphate (**8**). In the course of the latter transformation, C-1 of mevalonte, corresponding to the carboxyl carbon of one of the acetate precursors, is lost, thereby giving rise to farnesyl pyrophosphate in which six of the original acetate units have remained intact while three acetate-derived carbons have lost their original labeled neighbor (Fig. 5). The latter carbons appear simply as enhanced singlets in the ^{13}C-NMR spectra of all derived metabolites, while intact acetate pairs give rise to enhanced and coupled doublets, centered around the natural abundance singlets. The fact that six sets of such doublets appeared in the spectra of both **1** and **2** indicated that there had been no additional cleavage of bonds derived from individual acetate precursors. On the other hand, the formation of quadrone and terrecyclic acid clearly involves one or more rearrangements of the parent farnesyl skeleton. Cleavage of bonds between distinct acetate units cannot be detected by incorporation of multiple labeled acetate precursors and instead requires incorporation of more advanced intermediates carrying labels across bonds derived from distinct

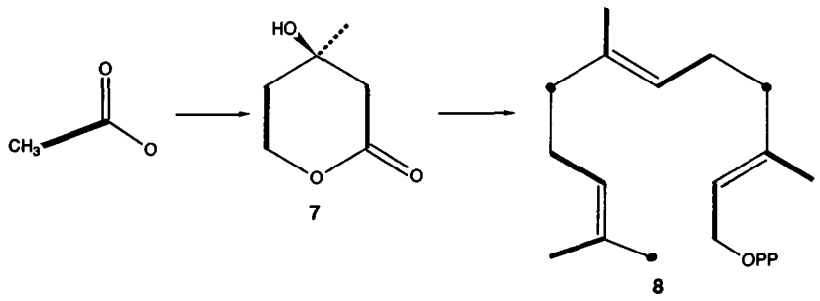

FIG. 5. Derivation of farnesyl pyrophosphate from [1,2-$^{13}C_2$]acetate.

acetate units. To identify the relevant bond-breaking reactions in the biosynthesis of quadrone, we therefore chose to carry out incorporations of [3,4-$^{13}C_2$]mevalonate, a precursor which we had used previously to elucidate the details of the carbon skeletal rearrangement leading to the formation of the fungal metabolite ovalicin (*30*).

The requisite sample of [3,4-$^{13}C_2$]mevalonolactone was prepared from [2-^{13}C] acetone and ethyl[2-^{13}C]acetate, as previously described (*30*). For feeding experiments the labeled substrate was diluted with two equivalents of unlabeled mevalonate and the mixture was hydrolyzed to the corresponding sodium salt by treatment with aqueous base. A small quantity of sodium [2-^{14}C]mevalonate was also added as an internal standard for a check on incorporation efficiency. To establish optimal conditions for incorporation of mevalonate, a variety of feeding regimens were tested using sodium [2-^{14}C]mevalonate in varying concentrations and administered at different stages in the fermentation. In contrast to the nearly 10-fold enrichments which had been possible with labeled acetate, we were never successful in obtaining apparent specific incorporations of mevalonate in excess of 2.0% per labeled site, as calculated from measured ^{14}C activities. Moreover, it was found that the best incorporation rates were achieved at the expense of product yield, since precursor concentrations in excess of 100 mg of mevalonate per 100-ml culture resulted in three- to fourfold reductions in the levels of quadrone recovered. Eventually, two feeding protocols were adopted, one involving administration of three equal portions of [3,4-$^{13}C_2$]mevalonate after 3, 4, and 5 days, followed by incubation for 2 additional days (method A), and a second (method B) in which the labeled precursor was added in one lot on Day 5 and fermentation carried out for a total of 11 days. In the latter case, predominantly terrecyclic acid was recovered, the accumulation of quadrone having been substantially reduced.

Analysis by ^{13}C NMR of terrecyclic acid obtained from each of the above feeding experiments revealed two pairs of enhanced and coupled doublets corresponding to the pairs C-11,13 and C-1,5 (Table 2 and Fig. 6). No additional couplings were observed, nor was it possible to detect enhancements of any additional peaks, including those assigned to the expected sites of enrichment, C-2 and C-8. Based on the ratio of the $^{13}C^{13}C$ satellites to the corresponding natural abundance peaks, the average enrichment per labeled site was calculated to be 0.1 atom percentage in excess of natural abundance, a level well below the limit for reliable detection by simple enhancement of singly labeled sites.[2] Analysis of a 4-mg sample of quadrone, obtained by incorporation method A, gave somewhat less clear cut but nevertheless complementary results. Both C-11 and C-13 displayed satellites, J_{CC} 33.2, consistent with the expected coupling of these two peaks. While the upfield portion of the C-5 satellite was readily discernible, the downfield signal was obscured by overlap with the resonance corresponding to C-2. The assigned coupling of 33 Hz was therefore estimated by doubling the observed separation (16.6 Hz) between the C-5 natural abundance peak and the upfield satellite. Unfortunately, the low signal to noise ratio of the C-1 resonance pre-

[2] By contrast, substantially higher enrichments of terrecyclic acid by labeled mevalonates (3–5%) have been reported by Beale *et al.* (*31*).

TABLE 2

INCORPORATION OF [3,4-$^{13}C_2$]MEVALONATE[a,b]

	Quadrone[c]		Terrecyclic acid[d]	
C	δ_C	J_{CC}, Hz	δ_C	J_{CC}, Hz
1	49.91	**[e]	54.98	46.5
5	52.29	33.2[f]	150.62	47.1
11	48.81	33.2	49.01	33.3
13	40.49	33.3	40.49	33.1

[a] Method A: A mixture of 0.080 g of [3,4-$^{13}C_2$]mevalonolactone (C-3, 99 at. %; C-4, 91 at. %) and 0.180 g of mevalonolactone was hydrolyzed to the acid sodium salt and administered in three equal portions at 72, 98, and 117 h, respectively, to eight 100-ml cultures, total incubation time 7 days; avg enrichment 0.1%.

[b] Method B: A mixture of 0.068 g of [3,4-$^{13}C_2$]mevalonolactone (99 at. %) and 0.131 g of mevalonolactone plus 1.45×10^7 dpm of [2-^{14}C]mevalonate was hydrolyzed to the acid sodium salt and administered in a single dose at 120 h to four 50-ml cultures, total incubation time 11 days; avg enrichment 0.1%.

[c] **1**: 4 mg (Method A), spectral width, 15,151 Hz, 32 K data points, 49.5°C pulse, acquisition time 1.08 s, 118,824 scans.

[d] **2**: spectral width 15,151 Hz, 32 K data points, 20°C pulse, acquisition time 1.08 s; 5 mg (method A), 54,270 scans; 48 mg (method B), 6938 scans.

[e] Satellites not detected.

[f] Calculated from position of upfield satellite signal.

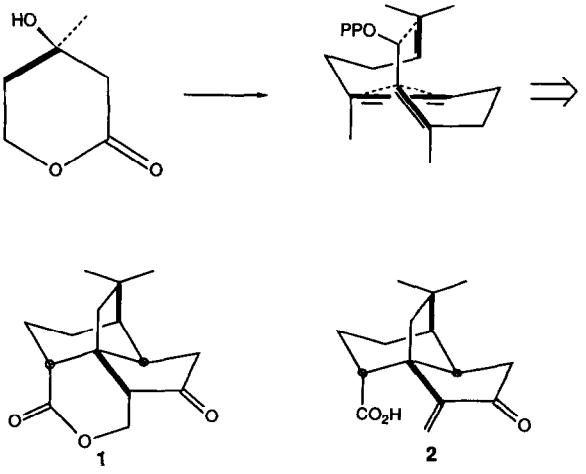

FIG. 6. Labeling of quadrone (**1**) and terrecyclic acid (**2**) by [3,4-$^{13}C_2$]mevalonate implicating cleavage of the 6,7-double bond of farnesyl pyrophosphate.

vented unambiguous detection of the expected complementary set of satellite signals.

DISCUSSION

The above-described labeling experiments establish conclusively the isoprenoid origins of quadrone and terrecyclic acid. Completely consistent results for the incorporation of [^{13}C]acetates into terrecyclic acid have been reported independently by the group of Hirota and Isogai (*32*) and that of Beale *et al.* (*31*). The observed labeling patterns consisting of six sets of enhanced and coupled doublets and three enhanced singlets in the spectra of both quadrone and terrecyclic acid derived from [1,2-$^{13}C_2$]acetate are completely in accord with the intermediacy of the universal sesquiterpene precursor, farnesyl pyrophosphate, itself derived from three units of mevalonate. Consistent with this picture are Rosazza's findings that [5-^{13}C]mevalonate labeled C-3, C-10, and C-12 of terrecyclic acid, while [2-^{13}C]mevalonate enhanced the peaks corresponding to C-4 and C-8, as well as the resonance which we now have assigned to C-15. As expected the latter three sites were identical with those which appeared as enhanced singlets in samples of **2** derived from [1,2-$^{13}C_2$]acetate. Moreover, knowing the absolute configuration of quadrone, the stereochemical course of the farnesyl pyrophosphate cyclization can be deduced, based on the unambiguous assignment of the geminal methyl resonances. Thus the fact that the *re*-methyl group, C-15, *cis* to the cyclopentanone ring, is derived from C-2 of mevalonate implies that cyclization of farnesyl pyrophosphate has taken place by electrophilic attack on the *si* face of the 10,11-double bond (Fig. 7). Interestingly, this conclusion indicates that the farnesyl precursor has been folded in the same absolute sense as the eventual quadrone product, a correlation which we have previously noted for a variety of dimethylcyclopentane sesquiterpenes (*23, 24*).

Although the cumulative data have established the origins of all the carbon atoms of both quadrone and terrecyclic acid and strongly support the postulated intermediacy of farnesyl pyrophosphate, the detailed mechanism of the key cyclization reaction remains unclear. Comparison of the structure of quadrone with that of farnesyl pyrophosphate leads to the recognition that formation of the tricyclic sesquiterpene necessarily requires cleavage of the 6,7-double bond of the acyclic precursor, a supposition consistent with our demonstration that incorporation of [3,4-$^{13}C_2$]mevalonate involves preservation of two mevalonate 3,4-bonds, with evident cleavage of the third.

Based on the results of our original acetate incorporation experiments, we previously proposed a mechanism for the formation of the quadrone skeleton from farnesyl pyrophosphate involving a pair of 1,2-hydride shifts, and leading to the formation of an as yet hypothetical sesquiterpene hydrocarbon (*25*). Although this postulated mechanism proved to be consistent with the results of our subsequent experiments with [3,4-$^{13}C_2$]mevalonate, it has been excluded by recently reported experiments which established that the methyl protons of acetate are retained at C-2 and C-8 of terrecyclic acid (*33*). An alternative cyclization mechanism, based

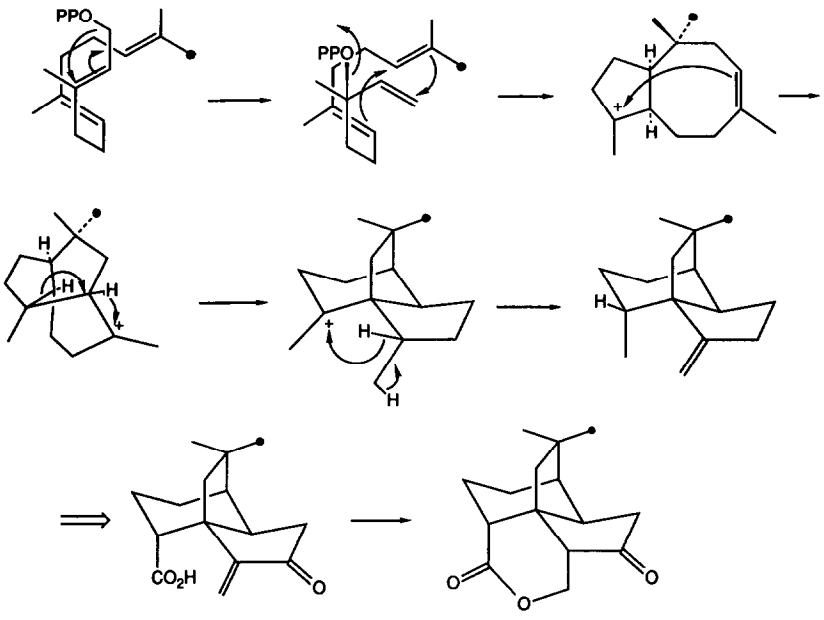

FIG. 7. Hypothetical cyclization of farnesyl pyrophosphate.

on that originally suggested by Hirota and Isogai and which accounts for all the available incorporation data, is illustrated in Fig. 7. Since the identity of the actual product of farnesyl pyrophosphate cyclization is as yet unknown, further differentiation between this and other mechanisms must await the isolation of additional metabolites as well as the results of further incorporation experiments.[3]

EXPERIMENTAL PROCEDURES

Materials and Methods. The preparation of [3,4-$^{13}C_2$]mevalonate has been previously described (*30*). A reference sample of quadrone was the gift of Dr. Matthew Suffness of the National Cancer Institute. Sodium [1-^{13}C]acetae [90 at.%] was obtained frm Stohler Isotope Chemicals. Sodium [1,2-$^{13}C_2$]acetate (99 at.%) and ethyl [2-^{13}C]acetate (91 at.%) were products of Merck, Sharpe, and Dohme. Ethyl [2-^{13}]acetate (99 at.%) and [2-^{13}C]acetone were purchased from Cambridge Isotope Laboratory. All other reagents and media components were reagent grade.

Flash chromatography on silica gel was carried out according to Still *et al.* (*35*). Radioactivity measurements were performed as previously described on Packard 3330 or Beckman 5801 scintillation counters (*22*). NMR spectra were recorded on

[3] Extensive examination of the nonpolar extracts of *A. terreus* mycelium has led to the identification of at least three sesquiterpene hydrocarbons, including (−)-(γ)-cadinene and aristolochene, but has to date failed to reveal any $C_{15}H_{24}$ metabolites related to quadrone (*34*).

a Bruker WM-250 spectrometer at 250 MHz (^1H) and 62.9 MHz (^{13}C). The ^1H-COSY spectrum of quadrone was acquired using 512 × 1024 data points in the f_1 and f_2 dimensions, respectively, with symmetrization of the resulting data set. Additional acquisition and data reduction parameters are provided in the headings for the various figures and tables.

Fermentation of A. terreus (1). Cultures of *A. terreus* NRRL 11,156 were obtained as lyophilized spore suspensions from Dr. D. T. Wicklow of the Northern Regional Research Center, USDA, in Peoria, Illinois. The rehydrated suspensions were used to inoculate potato-dextrose agar slants. A vegetative inoculum was prepared by adding 2.0 ml of sterile water to a slant and using 0.2 ml of the resulting spore suspension to inoculate a 500-ml flask holding 100 ml of sterile culture medium containing 40.0 g of Bacto–Dextrose, 3.0 g of Pharmamedia (Trader's), 1.0 g of soybean meal (Southern States Cooperative, Baltimore, Md.), 1.0 g of K_2HPO_4. 1.0 g $MgSO_4$. $7H_2O$, 0.001–0.002 g of $FeSO_4$, 0.5 g of NaCl, and 10 g of $CaCO_3$ per liter, pH adjusted to 7.0 with 3 M HCl. After incubation at 25°C and 250 rpm for 3 days, the vegetative culture was used to inoculate 100-ml fermenation cultures containing the identical medium (2 ml of incoulum per flask). Fermentations were normally carried out at 25°C and 250 rpm for 7 days. For long-term maintenance of *A. terreus*, 3-day-old vegetative inocula were mixed with sterile glycerol (2 : 1, v/v) and dispensed in 2.0-ml portions into Pro-Vials which were stored in an Orion ET-34 dewar over liquid nitrogen. Thereafter fresh vegetative inocula were routinely prepared by adding the contents of a freshly thawed Pro-Vial to 100 ml of vegetative medium. Production of quadrone and terrecyclic acid frequently varied, depending on the age of the inoculum and the Pharmamedia used.

Isolation of quadrone (1) and terrecyclic acid (2). At the end of the incubation period, the fermentation broths were separated from the mycelial mass, usually by filtration, and extracted with $CHCl_3$. In some cases, acidification and further extraction yielded additional quantities of terrecyclic acid, but in most instances the bulk of (**2**) was recovered during extraction of the neutral fraction. Initial separation of the terrecyclic acid and quadrone fractions was achieved by flash chromatography using 5 : 2 hexane–ethyl acetate, followed by 3 : 2 and 1 : 2 proportions of the same eluting solvents. Further purification was effected by preparative thin-layer chromatography on silica gel ($CHCl_3$–MeOH, 24 : 1; quadrone, R_f 0.55, 2–16 mg/liter; terrecyclic acid, R_f 0.35, 20–35 mg/liter). Frequently several purifications were necessary, especially for terrecyclic acid which retained persistent traces of unidentified impurities.

Incorporation of labeled precursors. Labeled precursors were dissolved in deionized water and added through a disposable sterile filtration unit to fermenting cultures at the intervals summarized in the tables.

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