phosphorylating agent of unusual nonselectivity can be formed in basic solution and, prior to its reaction with amino nitrogen or aromatic ring, is probably as "free" as any metaphosphate in solution.

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References and Notes

(1) A. J. Kirby and S. G. Warren, "The Organic Chemistry of Phosphorus", Elsevier, Amsterdam, 1967, p 281 ff; T. C. Bruice and S. Benkovic, "Bioorganic Mechanisms", Vol. 2, W. A. Benjamin, New York, N.Y., 1966, pp 22–25 and 157–159; S. J. Benkovic and K. J. Schray in "The Enzymes", Vol. VIII, 3rd ed, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1973, p 201; W. P. Jencks, "Catalysis in Chemistry and Enzymology", McGraw-Hill, New York, N.Y., 1969, pp 81–83, 112–115, 151, 160–161, and 608; W. W. Butcher and F. H. Westheimer, *J. Am. Chem. Soc.*, **77**, 2420 (1955); P. W. C. Barnard, C. A. Bunton, D. R. Llewellyn, K. G. Oldham, B. L. Silver, and C. A. Vernon, *Chem. Ind. (London)*, 760 (1955); A. R. Todd, *Proc. Natl. Acad. Sci. U.S.A.*, **45**, 1389 (1959); G. Di Sabato and W. P. Jencks, *J. Am. Chem. Soc.*, **83**, 4400 (1961); A. J. Kirby and A. G. Varvoglis, *ibid.*, **89**, 415 (1967); P. Haake and P. S. Ossip, *ibid.*, **93**, 6924 (1971); D. G. Gorenstein, *ibid.*, **94**, 2523 (1972); R. Kluger, *J. Org. Chem.*, **38**, 2721 (1973).
(2) C. H. Clapp and F. H. Westheimer, *J. Am. Chem. Soc.*, **96**, 6710 (1974); C. H. Clapp, A. Satterthwait, and F. H. Westheimer, *ibid.*, **97**, 6873 (1975).
(3) J. Rebek and F. Gaviña, *J. Am. Chem. Soc.*, **97**, 1591, 3221 (1975).
(4) E. Niecke and W. Flick, *Angew. Chem., Int. Ed. Engl.*, **13**, 134 (1974); O. J. Scherer and N. Kuhn, *Chem. Ber.*, **107**, 2123 (1974); N. T. Kulbach and O. J. Scherer, *Tetrahedron Lett.*, 2297 (1975); M. Regitz, H. Scherer, W. Illger, and H. Eckes, *Angew. Chem., Int. Ed. Engl.*, **12**, 1010 (1973); M. Regitz, A. Liedhegener, W. Anschütz, and H. Eckes, *Chem. Ber.*, **104**, 2177 (1971); M. Regitz, H. Scherer, and W. Anschütz, *Tetrahedron Lett.*, 753 (1970).
(5) J. B. Conant and A. A. Cook, *J. Am. Chem. Soc.*, **42**, 830 (1920); J. B. Conant and S. M. Pollack, *ibid.*, **43**, 1665 (1921); J. B. Conant and E. L. Jackson, *ibid.*, **46**, 1003 (1924); J. B. Conant and B. B. Coyne, *ibid.*, **44**, 2530 (1922).
(6) J. A. Maynard and J. M. Swan, *Aust. J. Chem.*, **16**, 596 (1963).
(7) G. L. Kenyon and F. H. Westheimer, *J. Am. Chem. Soc.*, **88**, 3557, 3561 (1966).
(8) A. R. Cook and D. I. Randall, *Nature (London)*, **218**, 974 (1968).
(9) B. G. Audley and B. L. Archer, *Chem. Ind. (London)*, 634 (1973).
(10) W. Vogt, *Tetrahedron Lett.*, 1281 (1970).
(11) F. Krolipfeiffer, *Justus Liebigs Ann. Chem.*, **430**, 161 (1923).
(12) T. Glonek and J. R. Van Waser, *J. Phys. Chem.*, **80**, 639 (1976).
(13) A. J. Kirby and W. P. Jencks, *J. Am. Chem. Soc.*, **87**, 3209 (1965).
(14) J. R. Cox, Jr., and O. B. Ramsay, *Chem. Rev.*, **64**, 317 (1964); A. J. Kirby and M. Younas, *J. Chem. Soc. B*, 1165 (1970); A. J. Khan and A. J. Kirby, *ibid.*, 1172 (1970).
(15) V. M. Clark and S. G. Warren, *Proc. Chem. Soc., London*, 178 (1963).
(16) T. Yamaoka, H. Hosaya, and S. Nagakura, *Tetrahedron*, **24**, 6203 (1968); **26**, 4125 (1970); M. Liler, *Adv. Phys. Org. Chem.*, **11**, 289–291, 356–357 (1975); D. P. Martinsen and S. E. Buttrill, Jr., *Org. Mass Spectrom.*, **11**, 762 (1976); T. Fujita and T. Nishioka, *Prog. Phys. Org. Chem.*, **12**, 49 (1976); R. G. Cavell and D. A. Allison, *J. Am. Chem. Soc.*, **99**, 4203 (1977); K. D. Summerhays, S. K. Pollack, R. W. Taft, and W. J. Hehre, *ibid.*, **99**, 4585 (1977).
(17) A. J. Kirby and A. G. Varvoglis, *J. Chem. Soc. B*, 135 (1968).
(18) A. F. Gerrard and N. K. Hamer, *J. Chem. Soc. B*, 539 (1968); 369 (1969).
(19) H. Weiner and R. A. Sneen, *J. Am. Chem. Soc.*, **87**, 287, 292 (1965); L. P. Hammett, "Physical Organic Chemistry", 2nd ed, McGraw-Hill, New York, N.Y., 1970, pp 160–161.

Biosynthesis of Cyclonerodiol

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Abstract: Sodium [2-^{14}C]mevalonate was incorporated into the cyclopentanoid sesquiterpene cyclonerodiol by cultures of *Gibberella fujikuroi*. Chemical degradation located the label equally at C-4, C-8, and C-12. A biosynthetic pathway consistent with the observed labeling pattern involves cyclization of nerolidyl pyrophosphate by addition of water across the vinyl and central double bonds.

Iridoid and derivative classes of cyclopentanoid monoterpenes are found in a large variety of higher plants and some insect species.[1] Typical structures are those for loganin (**1**),[2] the demonstrated precursor of the nontryptamine portion of the indole alkaloids,[3] and iridomyrmecin (**2**), a secretory component of the ant, *Iridomyrmex humilis*.[4]

In contrast to the widespread occurrence of these 1,2,3-substituted cyclopentane monoterpenes, only a handful of structurally related sesquiterpene metabolites is known, the majority being produced by higher fungi. Best studied are cyclonerodiol (**3**)[5-7] and two closely related substances, cyclonerodiol oxide (**4**)[5a] and cyclonerotriol (**5**).[5c,7] These metabolites are distinguished from the cyclopentanoid monoterpenes by oxygenation pattern and by having trans,trans ring stereochemistry.[8]

The biosynthesis of cyclonerodiol and cyclonerotriol has been studied by Hanson who fed [4,5-$^{13}C_2$]mevalonate to cultures of *Fusarium culmorum*.[9] The ^{13}C NMR spectra of each of the derived metabolites **3** and **5** showed 3 pairs of enhanced and coupled doublets corresponding to C-9 and C-10, C-1 and C-2, and C-5 and C-6 in cyclonerodiol and cyclonerotriol, respectively. These results established the intact incorporation of three molecules of mevalonate and were supported by feedings of various $^{3}H/^{14}C$-labeled mevalonates. In the latter experiments no significant changes were observed in $^{3}H/^{14}C$ ratio in going from precursor to product. Finally, feeding of biosynthetically labeled **3** to *F. culmorum* gave a 15% incorporation into cyclonerotriol.

In connection with our own interest in the stereochemistry of biosynthetic processes we have been studying the biosynthesis of cyclonerodiol. As a first step we have incorporated ^{14}C-labeled mevalonate and devised a degradation sequence

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Table I. Incorporation of Labeled Mevalonate[a] into Cyclonerodiol and Distribution of Activity

Compd	Sp act., dpm/mmol	Obsd %[b]	Theor %[b]
3	2.15×10^6	92[c]	100
6	2.33×10^6	100	100
7	1.61×10^6	69.3	66.7
8	1.59×10^6	68.1	66.7
10	1.57×10^6	67.2	66.7
15	0.05×10^6	2.3	0.0
16	0.795×10^6	34.2	33.3
16[d]	3.52×10^4	34.2	33.3
21	3.35×10^4	32.5	33.3
15a	0.06×10^4	0.6	0.0
17	0.780×10^6	33.5	33.3
17[d]	2.28×10^4	33.5	33.3
15c	0.18×10^4	3.3	0.0
21a	2.12×10^4	31.5	33.3
15d	0.02×10^4	0.3	0.0
15b[e]	0.15×10^4	1.4	0.0
17a	0.62×10^6	27	33.3

[a] Sodium [2-^{14}C]mevalonate, 2×10^8 dpm. [b] Based on bis(dinitrobenzoate) **6**. [c] Incorporation, 1.5%. [d] After dilution. [e] From Kuhn-Roth on **17**, 1.11×10^5 dpm/mmol.

to locate unambiguously the sites of labeling. Our results, which are completely consistent with and complement those of Hanson, are reported below.

Results

Sodium [2-^{14}C]mevalonate was administered to 5-day-old shaken cultures of *G. fujikuroi*. After an additional 2 days the cultures were harvested by filtration and extraction with ether. Cyclonerodiol was purified by chromatography of the concentrated extract on silica gel. The labeled metabolite was diluted to 0.195 g with inactive carrier and a portion of this material was converted to the bis(dinitrobenzoate) **6**[5b] which was recrystallized to constant activity. In separate experiments the percent incorporation varied from 1.1 to 2.5.

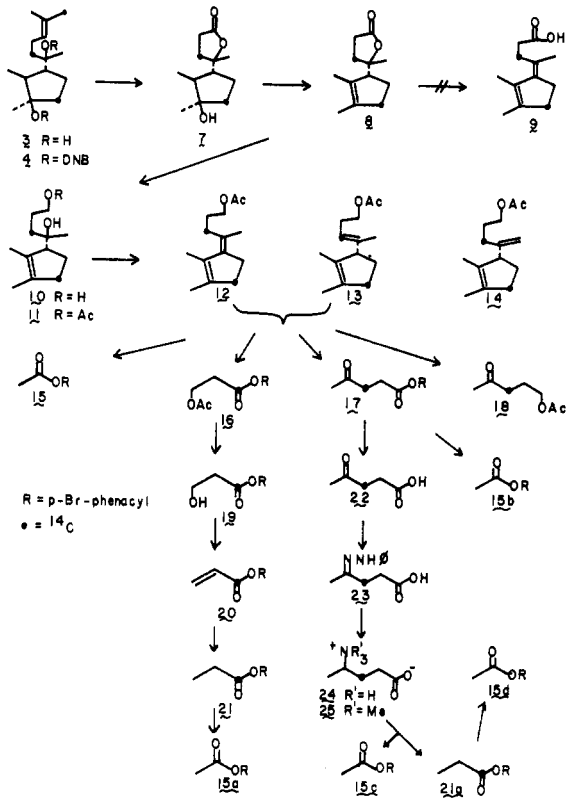
In order to establish the distribution of isotope within the labeled cyclonerodiol, the latter substance was subjected to a series of degradation reactions. Ozonolysis of **3** in ethyl acetate at −78 °C followed by treatment of the ozonide with Jones reagent gave the known norlactone **7**[5] which retained 69% of the total radioactivity. (Results of this and subsequent degradation reactions are summarized in Table I and illustrated in Scheme I.) Dehydration of **7** using thionyl chloride in pyridine gave a second known substance, the anhydrolactone **8**.[5] Attempts to convert **8** to the diene acid **9** under acidic (trifluoroacetic acid), basic (DBU, triethylamine), or dehydrating conditions (0.95 equiv of KOH followed by thionyl chloride–pyridine)[10] gave only recovered starting material. The anhydrolactone was therefore converted by lithium aluminum hydride reduction and subsequent acetylation to the olefinic diol monoacetate **11**. When **11** was treated with freshly distilled thionyl chloride in pyridine–methylene chloride at −78 °C a mixture of three diene acetates **12, 13,** and **14** was formed in a ratio of 4:4:1. Reaction at 0 °C gave predominantly trisubstituted olefin **13** and none of the conjugated diene **12**. The disubstituted olefin could be removed from the mixture by PLC on silver nitrate impregnated silica gel. Although in preliminary small-scale experiments on unlabeled material, diene **12** could be isolated by silver nitrate PLC and identified by its characteristic NMR (no olefinic proton) and UV spectra (λ_{max} 253 nm, ethanol), in practice it was found most convenient to use the mixture of **12** and **13** for the subsequent oxidation step.

In the event, **12** and **13** were treated with ruthenium dioxide–sodium periodate according to Schooley's modification[11] of the Piatak procedure,[12] and the reaction mixture was separated into acidic and neutral fractions. The potassium salts of the acidic products were treated with *p*-bromophenacyl bromide in the presence of 18-crown-6[13] and the resulting esters were separated by PLC, yielding the *p*-bromophenacyl esters of acetic, β-acetoxypropionic, and levulinic acids, **15, 16,** and **17,** respectively. The bulk of each of these derivatives was recrystallized to constant activity. The acetoxypropionic acid and levulinic acid fragments, derived from the side chain of **13** and the cyclopentane ring of **12,** respectively, contained the expected one-third of the total cyclonerodiol activity, while the acetic acid (from C-1 and C-2 of **12** plus a small amount from hydrolysis of the various acetate esters) was devoid of activity. The neutral fraction was shown by gas chromatography to contain predominantly 5-acetoxy-2-pentanone (**18**), identical with a sample prepared from levulinic acid. The keto acetate **18** could be converted to crystalline **17a** by lithium aluminum hydride reduction followed by Jones oxidation and formation of the *p*-bromophenacyl ester. The sample thus obtained contained slightly less than the theoretical activity and further purification was precluded by the small amount of material available. An alternative approach in which the semicarbazone was formed from PLC-purified **18** also gave low radioactivity values.

Returning to the acidic fragments, the remaining samples of **16** and **17** were each diluted with inactive carrier to 55 and 108 mg, respectively, and a portion of the diluted esters recrystallized to constant activity. The acetoxypropionate derivative was converted to the *p*-bromophenacyl ester of propionic acid (**21**) by hydrolysis of the acetate function, elimination of water via the derived mesylate, and reduction of the acrylate ester **20** over palladium/carbon. Kuhn–Roth oxidation of **21** gave inactive acetic acid, characterized as its *p*-bromophenacyl ester (**15a**). The degradation sequence locates the ^{14}C label in the carboxyl carbon of **21** and, by extension, at C-8 of cyclonerodiol.

A portion of the levulinic acid *p*-bromophenacyl ester (**17**) was subjected to Kuhn–Roth oxidation. The derived acetic acid (as the *p*-bromophenacyl ester (**15b**)) was devoid of activity.

Scheme I

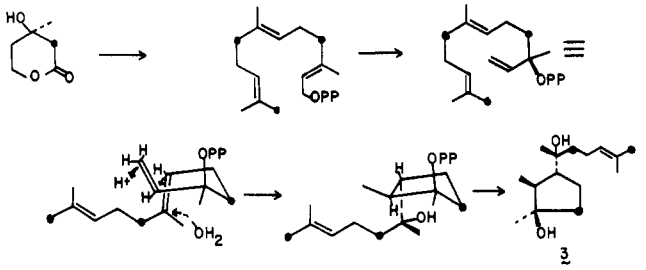


The remainder of the diluted **17** was converted to levulinic acid (**22**) by selective removal of the phenacyl ester with zinc in acetic acid.[14] Degradation of levulinic acid according to the method developed by Cornforth[15] (formation of phenylhydrazone, aluminum amalgam reduction to the amine, exhaustive methylation, and fusion with potassium hydroxide) gave acetic and propionic acids. Although the two acids could be separated by phase-partition chromatography on Celite, it was more convenient to convert the mixture to the corresponding *p*-bromophenacyl esters (**15c** and **21a**, respectively) which were readily separated by PLC. The propionic ester fragment (**21a**), which contained essentially all the ^{14}C activity, was oxidized by the Kuhn-Roth procedure to give inactive acetic acid. This latter sequence establishes the presence of label at C-3 of levulinic acid and therefore at C-4 of cyclonerodiol.

Discussion

A biosynthetic scheme to explain the observed labeling pattern in cyclonerodiol is illustrated in Scheme II. According

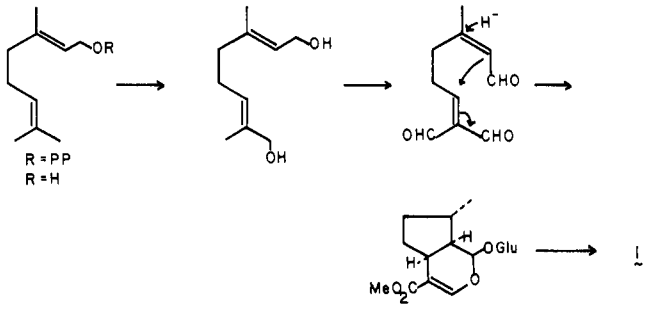
Scheme II



to this proposal mevalonate is converted in the normal fashion to *trans,trans*-farnesyl pyrophosphate[16] which in turn is transformed to nerolidyl pyrophosphate. Folding of the nerolidyl skeleton as indicated and overall trans-coplanar addition of water across the two double bonds as well as pyrophosphate hydrolysis will produce cyclonerodiol having the known absolute configuration. The concomitant hydrolysis of the pyrophosphate ester may provide a driving force for the double bond hydration step in a manner analogous to the hydrolysis of citryl-CoA in the reaction catalyzed by citrate synthetase.[17] Free nerolidol does not appear to be an intermediate in the biosynthetic sequence.[18]

The proposed biosynthetic pathway may be compared with that which has been established in part for the iridoid monoterpenes (Scheme III).[19] In the latter case, geraniol, derived

Scheme III



from hydrolysis of its pyrophosphate, undergoes a series of oxidations prior to a Michael-type cyclization step. Comparison of the oxidation patterns in cyclonerodiol and in the iridoid family suggests distinct biosynthetic mechanisms for the key cyclization of the respective acyclic prenyl precursors. Further work designed to clarify these intermediate steps and to answer some of the intriguing stereochemical questions inherent in the proposed pathway is in progress.

Experimental Section

Instrumentation. Proton NMR were obtained on Varian A60A and Bruker WP-60 spectrometers. ^1H NMR shifts are expressed in parts per million downfield from internal tetramethylsilane ($\delta = 0$). Infrared spectra were taken using a Perkin-Elmer Model 257 grating spectrophotometer and UV spectra were recorded on a Cary 14 instrument. Melting points were taken in unsealed melting point capillaries in a Hoover melting point apparatus and are uncorrected. Carbon-14 activity was measured using a Packard 3330 liquid scintillation counter and 10-mL toluene solutions containing 7.20 g of Bu-PBD and 0.45 g of PBBO per L of toluene. All solid samples were recrystallized at least three times to constant activity and counted two or more times to greater than 10 000 counts. Fungal fermentations were carried out in a New Brunswick Scientific G25 gyrotory incubator shaker.

Materials. DL-[2-^{14}C]Mevalonic acid lactone (17.5 mCi/mmol) was obtained from Amersham/Searle and converted to the sodium salt by hydrolysis with 1 equiv of 0.01 N aqueous sodium hydroxide. Strains of *G. Fujikuroi* were purchased from the American Type Culture Collection (ATCC 12616) as agar slants. Corn steep liquor was a gift of A. E. Staley Manufacturing Co.

Thionyl chloride was purified by distillation from quinoline and then redistillation from sulfur. Pyridine was distilled from barium oxide. Tetrahydrofuran was freshly distilled from lithium aluminum hydride.

Preparative layer chromatography (PLC) was carried out using 20 × 20 cm plates, 2 mm thickness, of Merck silica gel PF-254 buffered to pH 7, or on Merck precoated silica gel F-254 TLC plates, 0.25 mm thickness.

Incorporation of Labeled Mevalonate. *Gibberella fujikuroi* (ATCC 12616) was maintained on potato dextrose agar slants. A nutrient solution consisting of 200 g of dextrose, 6.0 g of ammonium succinate, 0.5 g of potassium dihydrogen phosphate, 0.2 g of magnesium sulfate heptahydrate, 0.2 g of potassium sulfate, and 6.0 mL of Corn Steep liquor per L of distilled water[5] was distributed in 10 500-mL DeLong flasks (100 mL per flask) fitted with Morton closures. The contents were autoclaved at 120 °C for 20 min.

Each flask was inoculated with a 5-mm plug of mycelium of *G. fujikuroi* and then incubated at 27 °C and 220 rpm in an incubator-shaker. After 5 days, an aqueous solution of sodium DL-[2-^{14}C]-mevalonate (2×10^8 dpm) was distributed over the ten flasks by sterile filtration (Swinnex-13 Millipore filter unit). After an additional 2 days a small quantity of Celite was added to each flask and the cultures were harvested by filtration. The filtrate was extracted with three 500-mL portions of ether and the combined organic extract was washed with 100 mL of water, 50 mL of 5% sodium hydroxide, 50 mL of water, and saturated sodium chloride. The ether extract was dried over sodium sulfate and concentrated under reduced pressure. The crude residue was partially purified by PLC (ether; R_f 0.3) and the resultant cyclonerodiol repurified by a second PLC (methylene chloride–ether, 3:1; two developments, R_f 0.25) to yield 14 mg of cyclonerodiol: NMR ($CDCl_3$) δ 1.03 (d, J = 7 Hz, CH_3CH, 3 H), 1.16 (s, CH_3), 1.25 (s, CH_3, 3 H), 1.65 (s, CH_3) and 1.70 (s, CH_3) (total 6 H), 1.35–2.3 (m, 12 H), 5.10 (t, J = 7 Hz, CH=, 1 H); IR ν_{max} ($CHCl_3$) 3610, 3450 cm^{-1} (OH).

The labeled cyclonerodiol was diluted with inactive carrier to a total of 195 mg for further degradation.

Cyclonerodiol Bis(3,5-dinitrobenzoate). A mixture of 7.5 mg of cyclonerodiol and 21 mg of 3,5-dinitrobenzoyl chloride in 1 mL of dry methylene chloride containing 0.2 mL of pyridine was stirred at room temperature for 8 days. Water was added to the reaction mixture followed by ether. The ether was washed successively with 5% sulfuric acid, saturated potassium bicarbonate, and saturated sodium chloride, dried over sodium sulfate, and concentrated under reduced pressure. Purifcation by PLC (methylene chloride–ether, 3:1; R_f 0.82) followed by recrystallization from carbon tetrachloride gave needles: mp 141–143 °C (lit.[5b] mp 141–143 °C); NMR ($CDCl_3$) δ 1.28 (d, J = 7 Hz, CH_3CH, 3 H), 1.67 (br s, CH_3, 6 H), 1.73 (s, CH_3, 3 H), 1.78 (s, CH_3, 3 H), 1.6–3.2 (m, 10 H), 5.16 (t, J = 7 Hz, CH=, 1 H), 9.16 (aromatic, 6 H); IR ν_{max} ($CHCl_3$) 1720 cm^{-1} (C=O).

The radioactive sample of **6** was recrystallized to constant activity (2.33×10^6 dpm/mmol) and this value was used as a reference standard for subsequent degradations. Radioactivity data for all degradation products are summarized in Table I.

Ozonolysis of Cyclonerodiol to Hydroxylactone 7.[5] Cyclonerodiol

(177 mg, 0.74 mmol) in 50 mL of methylene chloride was treated with excess ozone at −78 °C. After the starting material had been consumed the solution was warmed to 0 °C and flushed with nitrogen to remove excess ozone. After evaporation of the solvent the resulting white residue was taken up in 30 mL of acetone and treated dropwise with 8 N Jones reagent at room temperature until the orange color persisted. After destruction of excess oxidant with a few drops of 2-propanol, the solvent was evaporated and the residue was dissolved in 10 mL of water and extracted with ethyl acetate which in turn was concentrated to dryness. PLC (ether, R_f 0.2) gave 0.094 g of hydroxylactone (**7**), mp 81.5–83 °C (lit.[5] mp 82–83 °C), a portion of which was recrystallized from chloroform to constant activity: NMR ($CDCl_3$) δ 1.05 (d, J = 6 Hz, CH_3CH, 3 H), 1.26 (s, CH_3, 3 H), 1.38 (s, CH_3, 3 H), 1.45–2.3 (m, 9 H), 2.4–2.9 (m, CH_2CO, 2 H); IR ν_{max} ($CHCl_3$) 3600, 3500 (OH), 1765 cm^{-1} (C=O).

Dehydration of Hydroxylactone 7 to Olefinic Lactone 8.[5] The hydroxylactone (**7**) (0.089 g, 0.42 mmol) in 0.2 mL of pyridine and 5 mL of methylene chloride was slowly treated with freshly distilled thionyl chloride solution (0.1 mL of thionyl chloride in 1 mL of methylene chloride) at −10 °C. The reaction mixture was stirred vigorously at the same temperature for another hour and then poured into 5 mL of 1 N hydrochloric acid at 0 °C. The product was extracted with ethyl acetate which was washed with 5% sodium bicarbonate and saturated sodium chloride, then dried over sodium sulfate. The concentrated extract was purified by PLC (ether, R_f 0.6) to give 0.047 g of **8**: NMR ($CDCl_3$) δ 1.33 (s, CH_3, 3 H), 1.65 (br s, CH_3, 6 H), 1.65–3.1 (m, 9 H); IR ν_{max} ($CHCl_3$) 1778 cm^{-1} (C=O).

Reduction of Lactone 8. Lactone **8** (0.015 g, 0.077 mmol) was treated with 0.010 g (0.26 mmol) of lithium aluminum hydride in 7.0 mL of tetrahydrofuran at room temperature for 3 h. After addition of a drop of water and a drop of 1 N sodium hydroxide and removal of the precipitate by filtration, the crude product was purified by PLC (ethyl acetate, R_f 0.21) to yield 0.0145 g of diol **10**: mp 93–96 °C; NMR ($CDCl_3$) δ 1.15 (s, CH_3, 3 H), 1.67 (br s, CH_3, 6 H), 1.5–3.0 (m, 11 H), 3.68 (t, J = 6.5 Hz, CH_2O, 2 H); IR ν_{max} ($CHCl_3$) 3600, 3450 cm^{-1} (OH). Anal. ($C_{12}H_{22}O_2$) C, 72.86; H, 11.33.

Diol Monoacetate (11). The diol **10** (0.015 g, 0.075 mmol) was treated with 0.05 mL of acetic anhydride and 0.1 mL of pyridine in 3 mL of methylene chloride at room temperature overnight. Subsequent addition of water, followed by extraction with ethyl acetate and drying over sodium sulfate, gave, after solvent evaporation and PLC purification, 0.017 g of monoacetate **11**: NMR ($CDCl_3$) δ 1.15 (s, CH_3, 3 H), 1.67 (br s, CH_3, 6 H), 2.07 (s, CH_3CO, 3 H), superimposed on 1.5–3.1 (m, 10 H), 4.12 (t, J = 6.5 Hz, CH_2O, 2 H); IR ν_{max} ($CHCl_3$) 3620, 3500 (OH), 1730 cm^{-1} (C=O). Anal. Calcd for $C_{14}H_{22}O_{22}$ ($M^+ - H_2O$): mol wt 222.1620. Found: mol wt 222.1610.

Dehydration of Diol Monoacetate (11). Diol monoacetate (**11**) (5 mg, 21 μmol) was dissolved in 2 mL of methylene chloride containing 0.1 mL of pyridine and cooled to −78 °C. To this mixture was added 3.6 mg (30 μmol) of freshly distilled thionyl chloride in methylene chloride. After 1 h the reaction mixture was poured into 1 mL of 1 N hydrochloric acid at 0 °C and extracted with ether. The ether layer was washed with 5% sodium bicarbonate followed by saturated sodium chloride and then dried over sodium sulfate and concentrated under vacuum. Preliminary separation by PLC (methylene chloride–ether, 2:1; R_f 0.73) gave 3 mg of a mixture containing the three isomeric dienes **12, 13,** and **14** in a ratio of 4:4:1 as determined by NMR (diene **12** has no olefinic proton, **13** has an olefinic H at δ 5.16 (t), while **14** has two exo methylene hydrogens at δ 4.85 (br s)). The mixture was chromatographed on silver nitrate impregnated silica gel TLC plates (prepared by dipping for 5 s into a freshly prepared 3% solution of silver nitrate in 1:1 ethanol–acetonitrile, then air drying for 30 min) (benzene: R_f (**12**) 0.44, R_f (**13**) 0.44, R_f (**14**) 0.37) to remove the *exo*-olefin. The mixture of **12** and **13** was used for the subsequent oxidation step.

In a small scale experiment **12** was separated from **13** by repeated PLC (silver nitrate/silica gel; benzene) and careful excision of the two zones. The conjugated diene **12** was the less polar component: NMR ($CDCl_3$) δ 1.25 and 1.65 (allylic CH_3), 2.05 (s, CH_3CO) superimposed on 1.5–2.6 (m, total ca. 17 H), 4.15 (t, J = 7 Hz, CH_2O, 2 H); IR ν_{max} ($CHCl_3$) 1730 cm^{-1} (C=O); UV ν_{max} (EtOH) 253. **13**: NMR ($CDCl_3$) δ 1.45 (br s, allylic CH_3, 6 H), 1.67 (br s, allylic CH_3, 3 H), 2.05 (s, CH_3CO, 3 H) superimposed on 1.2–2.5 (m, 6 H), 2.8–3.3 (m, =CCH=, 1 H), 4.1 (t, J = 7 Hz, CH_2O, 2 H), 5.2 (t, J = 7 Hz, CH=, 1 H); IR ν_{max} ($CHCl_3$) 1730 cm^{-1} (C=O).

Ruthenium Tetroxide Oxidation of the Diene Mixture.[11,12] A mixture of **12** and **13** (2 mg, 9 μmol, ca. 1:1) in 0.2 mL of *tert*-butyl alcohol was treated with 1 mg of ruthenium dioxide, 40 mg of sodium periodate, and 1.4 mg of periodic acid in 0.2 mL of water. After 1 h an additional 20 mg of sodium periodate in 0.4 mL of water was added and after another 1 h 20 mg of sodium periodate and 1 mg of ruthenium dioxide were added to the reaction mixture which was then stirred overnight. The reaction was terminated by addition of a few drops of 2-propanol until the solution was colorless with black ruthenium dioxide powder dispersed or attached to the inner surface of the flask. Water (1 mL) was added followed by 5 drops of saturated potassium carbonate solution. The reaction mixture was extracted with six 1-mL portions of ether and the combined ethereal extract was set aside at 0 °C. The aqueous layer was directly evaporated to a dry residue containing the potassium salts of levulinic, β-acetoxypropionic, and acetic acids. To this mixture was added 3 mL of acetonitrile containing 2 mg of 18-crown-6 and 20 mg of α-*p*-dibromoacetophenone and the reaction was stirred at room temperature overnight.[13] After filtration to remove potassium bromide, the solution was evaporated and purified by PLC. The acetate derivative **15** was first separated from **16** and **17** (benzene–hexane–ethyl acetate, 5:5:1; R_f (**15**) 0.28, R_f (**16**) 0.11, R_f (**17**) 0.07). The mixture of **16** and **17** was separated by a second PLC system (methylene chloride–ether, 5:1; R_f (**17**) 0.48, R_f (**16**) 0.59): yield, **17,** 0.4–0.7 mg; **16,** 0.3–0.5 mg; **15,** 0.5–0.7 mg. The sequence of dehydration and oxidation was repeated several times and the combined phenacyl esters were each recrystallized from methylene chloride–pentane to constant activity, portions of **16** and **17** being reserved for dilution with inactive carrier and further degradation.

***p*-Bromophenacyl β-acetoxypropionate (16):** mp 71.5–72 °C: NMR ($CDCl_3$) δ 2.07 (s, CH_3CO, 3 H), 2.83 (t, J = 6 Hz, CH_2CO, 2 H), 4.42 (t, J = 6 Hz, CH_2O, 2 H), 5.33 (s, OCH_2CO, 2 H) 7.7 (m, aromatic, 4 H); IR ν_{max} ($CHCl_3$) 1735, 1702 (C=O), 1586 cm^{-1} (aromatic). Anal. ($C_{13}H_{13}O_5Br$) C, 47.53; H, 4.07.

***p*-Bromophenacyl levulinate (17):** mp 83–84 °C (lit.[20] mp 84 °C); NMR ($CDCl_3$) δ 2.2 (s, CH_3, 3 H), 2.62 (br s, CH_2, 4 H), 5.3 (s, OCH_2CO, 2 H), 7.7 (m, aromatic, 4 H); IR ν_{max} ($CHCl_3$) 1745, 1720, 1710 (C=O), 1590 cm^{-1} (aromatic).

***p*-Bromophenacyl acetate (15):** mp 84–85 °C (lit.[21] mp 86.0 °C); NMR ($CDCl_3$) δ 2.2 (s, CH_3, 3 H), 5.28 (s, OCH_2CO, 2 H), 7.7 (m, aromatic, 4 H); IR ν_{max} ($CHCl_3$) 1750, 1703 (C=O), 1590 cm^{-1} (aromatic).

Degradation of β-Acetoxypropionic Acid *p*-Bromophenacyl Ester (16). A portion of the β-acetoxypropionic acid *p*-bromophenacyl ester was diluted with inactive carrier to 0.055 g and a small quantity was recrystallized to constant activity.

(a) *p*-Bromophenacyl β-Hydroxypropionate (19). *p*-Bromophenacyl β-acetoxypropionate (0.029 g, 0.088 mmol) in 2 mL of methanol was treated with three drops of saturated potassium carbonate for 1 h. The solvent was evaporated and the residue treated with 35 mg of α-*p*-dibromoacetophenone and 4 mg of 18-crown-6 in 5 mL of acetonitrile overnight. Evaporation of the solvent and PLC (ether; R_f 0.31) gave 16 mg of β-hydroxypropionic acid *p*-bromophenacyl ester (**19**): mp 86–88.5 °C; NMR ($CDCl_3$) δ 2.75 (t, J = 6 Hz, CH_2CO, 2 H), 3.05 (m, OH, 1 H), 4.0 (m, CH_2O, 2 H), 5.36 (s, OCH_2CO, 2 H), 7.71 (m, aromatic, 4 H); IR ν_{max} ($CHCl_3$) 3480 (OH), 1735 (C=O), 1688 (H-bonded ester), 1590 cm^{-1} (aromatic).

(b) *p*-Bromophenacyl Acrylate (20). β-Hydroxypropionic acid *p*-bromophenacyl ester (**19**) (20 mg, 0.070 mmol) was dissolved in 2 mL of methylene chloride containing 12 mg of triethylamine, the solution was cooled to 0 °C, and 10.3 mg of methanesulfonyl chloride in 1 mL of methylene chloride was added slowly. After 1 h at 0 °C, the solvent was evaporated and the residue taken up in 3 mL of chloroform containing 60 mg of triethylamine. The mixture was refluxed for 3 h, the solvent was evaporated, and the crude product was purified by PLC (ether–methylene chloride, 1:1; R_f 0.56) to yield 12 mg of **20:** mp 68–68.5 °C: NMR ($CDCl_3$) δ 5.38 (s, CH_2CO, 2 H), 5.95 (m, vinyl, 1 H), 6.37 (m, vinyl, 2 H), 7.71 (m, aromatic, 4 H); IR ν_{max} ($CHCl_3$) 1728, 1700 (C=O), 1685 cm^{-1} (aromatic). Anal. ($C_{11}H_9O_3Br$) C, 49.31; H, 3.42.

(c) *p*-Bromophenacyl Propionate (21). *p*-Bromophenacyl acrylate (28 mg, 0.10 mmol) was reduced with 2.6 mL (0.11 mmol) of hydrogen over 4 mg of 5% palladium on charcoal in 5 mL of ethanol. Filtration to remove the catalyst and evaporation of the solvent followed by PLC (benzene–hexane–ethyl acetate, 5:5:1; R_f 0.38) gave *p*-bromophenacyl propionate (**21**) (27 mg).

(d) Kuhn-Roth Oxidation of *p*-Bromophenacyl Propionate.[22] *p*-Bromophenacyl propionate (**21**) (5 mg) was heated in an oil bath at 165 °C for 1.5 h with 4 mL of oxidizing reagent prepared from 16.7 g of chromium trioxide, 100 mL of water, and 25 mL of concentrated sulfuric acid. The reaction mixture was cooled to room temperature and the apparatus set up for steam distillation of the acetic acid using the flame from a bunsen burner. The distillate was titrated with 0.0078 N potassium hydroxide (phenolphthalein indicator) and the potassium acetate was treated with α,*p*-dibromoacetophenone to form *p*-bromophenacyl acetate (**15a**) as described above.

Degradation of Levulinic Acid *p*-Bromophenacyl Ester. A portion of **17** was diluted with inactive carrier to 0.108 g and a small quantity was recrystallized to constant activity.

(a) Cleavage of the Phenacyl Ester.[14] Levulinic acid *p*-bromophenacyl ester (**17**) (0.043 g, 0.14 mmol) was treated with 100 mg of ether-washed zinc dust in 1.5 mL of acetic acid at room temperature overnight. Unreacted zinc and zinc oxide were removed by filtration and the acetic acid was removed under reduced pressure. PLC (ethyl acetate-benzene, 1:1; collect band, R_f 0.0-0.3) gave 14 mg of levulinic acid (22).

(b) Levulinic Acid Phenylhydrazone (23).[15] Phenylhydrazine (0.02 mL) in acetic acid (0.06 mL) was added to a solution of 22 mg (0.19 mmol) of levulinic acid in 0.4 mL of water over 10 min. The reaction mixture was stirred vigorously for 1 h. The supernatant was separated by pipet and the precipitate was washed thoroughly with cold water (5 mL) to give 37 mg of **23:** NMR ($CDCl_3$) δ 1.82 (s, CH_3, 3 H), 2.65 (t, J = 2.5 Hz, CH_2, 4 H), 7.08 (m, aromatic, 5 H), 12.8 (br s, OH, 1 H); IR ν_{max} ($CHCl_3$) 2400-3600 (COOH), 1745 (C=O), 1635 cm^{-1} (aromatic).

(c) 4-Aminopentanoic Acid (24).[15] Levulinic acid phenylhydrazone (**23**) (37 mg, 0.18 mmol) in 5 mL of 80% aqueous ethanol was treated with freshly prepared amalgamated aluminum (generated by dipping small pieces of aluminum foil (0.06 g) in 1 N sodium hydroxide to obtain a clean surface, rinsing with water, and treatment with 5% mercuric chloride until the entire surface became shiny). The reaction mixture was stirred overnight following which the gray residue was separated by centrifugation and carefully rinsed with water. The aqueous layer was evaporated under reduced pressure to an oily residue which crystallized after rinsing with ether to remove aniline: yield (**24**), 16.5 mg; NMR (D_2O) δ 1.2 (d, J = 6.5 Hz, CH_3, 3 H), 1.80 (m, CH_2, 2 H), 2.1 (m, CH_2, 2 H), 3.3 (m, CH, 1 H).

(d) 4-Aminopentanoic Acid Methylbetaine (25).[15] 4-Aminopentanoic acid (**24**) (16.5 mg, 0.14 mmol) was mixed with 0.25 g of silver oxide in 1.2 mL of methanol. After 5 min, 0.05 mL of methyl iodide was added and the mixture was stirred for 40 h. The precipitate was removed by filtration and rinsed with 10 mL of methanol. The combined methanolic solutions were evaporated to dryness and washed thoroughly with sufficient chloroform to remove side products. The remaining white solid betaine was dried under high vacuum: **25,** 6 mg; NMR (D_2O) δ 1.43 (d, J = 7.2 Hz, CH_3C, 3 H), 2.32 (m, 4 H), 3.12 (s, CH_3N, 9 H). Additional betaine could be obtained by recycling the chloroform washings (overall 25% yield).

(e) Potassium Hydroxide Fusion of Betaine.[15] The betaine (**25**) (15 mg, 0.09 mmol) was mixed with 0.3 g of potassium hydroxide and heated in a sealed bomb tube under nitrogen at 350 °C for 10 min. The contents of the tube were transferred to a flask by dissolving in a minimum amount of water, the pH was adjusted to ca. 8 with 1 N HCl, and the water was evaporated. The potassium salts of acetic and propionic acids were converted to their respective *p*-bromophenacyl esters (**15c** and **21a**) by the procedure described above. The esters were separated by PLC (benzene-hexane-ethyl acetate, 5:5:1; R_f (**15c**) 0.25, R_f (**21a**) (0.38) and recrystallized to constant activity. Propionic acid *p*-bromophenacyl ester (**21a**): 7.6 mg; mp 62-63.5 °C (lit.[21] mp 63.4 °C). Acetic acid p-bromophenacyl ester (**15c**): 9.8 mg.

(f) Kuhn-Roth Oxidation of *p*-Bromophenacyl Propionate (21a). *p*-Bromophenacyl propionate (**21**) (5 mg) from the above degradation sequence was subjected to Kuhn-Roth oxidation to give inactive acetic acid, isolated as the *p*-bromophenacyl ester (**15d**).

(g) Kuhn-Roth Oxidation of *p*-Bromophenacyl Levulinate. *p*-Bromophenacyl levulinate (6 mg, diluted to 1.11×10^5 dpm/mmol) was subjected to Kuhn-Roth oxidation and the resultant acetic acid isolated as the *p*-bromophenacyl ester (**15b**).

5-Acetoxy-2-butanone. Authentic 5-acetoxy-2-butanone was prepared by Jones oxidation of pentane-1,4-diol obtained by lithium aluminum hydride reduction of methyl levulinate. Gas chromatographic analysis of the neutral extract of the labeled diene oxidation indicated that **18** was the major volatile component (6 ft; 5% Carbowax; 150 °C; t_R = 2.37 min).

Conversion of Neutral Oxidation Products to *p*-Bromophenacyl Levulinate (17a). The combined ethereal extracts from the oxidation of **12** and **13** were evaporated and then treated with 10 mg of lithium aluminum hydride in 10 mL of tetrahydrofuran. Addition of 3 drops of water followed by 3 drops of sodium hydroxide, centrifugation to remove the precipitate, and evaporation of the solvent gave pentane-1,4-diol which was treated with 8 N Jones reagent in acetone at room temperature. The levulinic acid formed was isolated as the potassium salt and converted the *p*-bromophenacyl ester (**17a**) as previously described. TLC of twice-recrystallized material (0.3 mg) showed an unknown minor component with a similar R_f value.

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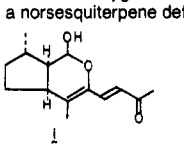
References and Notes

(1) W. I. Taylor and A. R. Battersby, Ed., "Cyclopentanoid Terpene Derivatives", Marcel Dekker, New York, N.Y., 1969.
(2) A. R. Battersby, E. S. Hall, and R. Southgate, *J. Chem. Soc. C,* 721 (1969); S. Brechbuhler-Bader, C. J. Coscia, P. Loew, Ch. von Szczepanski, and D. Arigoni, *Chem. Commun.,* 136 (1968).
(3) Inter alia: A. R. Battersby, R. S. Kapil, J. A. Martin, and L. Mo, *Chem. Commun.,* 133 (1968); P. Loew and D. Arigoni, *ibid.,* 137 (1968).
(4) G. W. K. Cavill, D. L. Ford, and H. D. Locksley, *Aust. J. Chem.,* **9,** 288 (1956); G. W. K. Cavill and H. D. Locksley, *ibid.,* **10,** 352 (1957); M. Pavan, *Chim. Ind. (Milan),* **37,** 625, 714 (1955).
(5) Isolation: (a) *Trichotecium roseum:* S. Nozoe, M. Goi, and N. Morisaki, *Tetrahedron Lett.,* 1293 (1970); (b) *Gibberella fujikuroi:* D. W. Pitel, G. P. Arsenault, and L. C. Vining, *J. Antibiot.,* **24,** 483 (1971); B. E. Cross, R. E. Markwell, and J. C. Stewart, *Tetrahedron,* **27,** 1663 (1971); (c) *Fusarium culmorum:* J. R. Hanson, P. B. Hitchcock, and R. Nyfeler, *J. Chem. Soc. Perkin Trans. 1,* 1586 (1975).
(6) Synthesis: S. Nozoe, M. Goi, and N. Morisaki, *Tetrahedron Lett.,* 3701 (1971).
(7) (a) Relative stereochemistry: ref 5c; (b) Absolute configuration: D. E. Cane and R. Iyengar, *Tetrahedron Lett.,* 3511 (1977).
(8) More closely related to the iridoids in oxygenation pattern and ring stereochemistry is gyrinidone (**1**), a norsesquiterpene defense substance isolated



1

from pygidial gland secretions of the whirligig beetle, *D. discolor* Aube: J. W. Wheeler, S. K. Oh, E. F. Benfield, and S. E. Neff, *J. Am. Chem. Soc.,* **94,** 7589 (1972).
(9) R. Evans, J. R. Hanson, and R. Nyfeler, *J. Chem. Soc., Perkin Trans. 1,* 1214 (1976).
(10) S. Sarel, Y. Shalon, and Y. Yanuka, *Chem. Commun.,* 80 (1970).
(11) D. A. Schooley, K. J. Judy, B. J. Bergot, M. S. Hall, and J. B. Siddall, *Proc. Natl. Acad. Sci. U.S.A.,* **70,** 2921 (1973).
(12) D. M. Piatak, H. B. Bhat, and E. Caspi, *J. Org. Chem.,* **34,** 112 (1969).
(13) H. D. Durst, M. Milano, E. J. Kokta, Jr., S. A. Corneily, and E. Grushka, *Anal. Chem.,* **47,** 1797 (1975).
(14) J. B. Hendrickson and C. Kandall, *Tetrahedron Lett.,* 343 (1970).
(15) J. W. Cornforth and G. Popjak, *Biochem. J.,* **58,** 403 (1954).
(16) Hanson has demonstrated incorporation of farnesyl pyrophosphate into cyclonerodiol.[9]
(17) H. Eggerer, U. Remberger, and C. Gruenewaelder, *Biochem. Z.,* **339,** 436 (1964).
(18) Cultures of *G. fujikuroi* failed to incorporate [12,13-^{14}C]nerolidol into cyclonerodiol (D. E. Cane and R. Iyengar, unpublished results). Hanson has made similar observations using *F. culmorum* and *T. roseum.*[9]
(19) Inter alia: Battersby et al., ref 2; Brechbuhler-Bader et al., ref 2; C. J. Coscia and R. Guarnaccia, *Chem. Commun.,* 138 (1968); S. Escher, P. Loew, and D. Arigoni, *ibid.,* 823 (1970); A. R. Battersby, S. H. Brown, and T. C. Payne, *ibid.,* 827 (1970); S. Escher, Dissertation, Eidgenössische Technische Hochschule, Zürich, No. 4887 (1972).
(20) Zvi Rappoport, Ed., "CRC Handbook of Tables for Organic Compound Identification", 3rd ed, CRC Press, Cleveland, Ohio, 1967, p 193.
(21) Ref 20, p 190.
(22) E. Wiesenberger, *Mikrochim. Acta,* **33,** 51 (1948); V. S. Pansare and S. Dev, *Tetrahedron,* **24,** 3767 (1968).