Isolation, Structure, and Radiochemical Synthesis of 3,6-Dimethyl-4-hydroxy-2-pyrone[1]

Terese E. Acker, Paul E. Brenneisen,[2] and Stuart W. Tanenbaum

Contribution from the Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, New York 10032.
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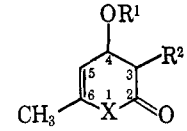
Abstract: A metabolite from *P. stipitatum*, $C_7H_8O_3$, was deduced to have the structure 3,6-dimethyl-4-hydroxy-2-pyrone from its spectral properties, and from conversion into its corresponding pyridone. This structure was fully confirmed by synthesis starting from ethyl acetoacetate and methylmalonic acid, a route which allows for the preparation of various isotopic species. The microscale radiochemical synthesis of this metabolite is exemplified by the preparation of 3,6-dimethyl-4-hydroxy-2-pyrone-3-C^{14}. The biogenesis of this oligoketide and its possible relationship to tropolone biosynthesis are discussed.

The biogenesis of secondary aromatic metabolites produced by the higher fungi has now been firmly established to result from the acetate–polymalonate pathway.[3] In contrast to what has recently been found in the case of the closely related biosynthetic pathway to avian or mammalian fatty acids,[4] fungal aromatic formation is still predicted to involve the obligatory participation of poly-β-ketide intermediates.[5–7] In spite of the fact that many organic analogies exemplify[8–10] the conversion of poly-β-ketides into aromatic derivatives, these compounds have yet to be detected in living systems presumably because of inherent instability. Equally disappointing have been attempts[5] to demonstrate incorporation of labeled lower polyketides into fungal metabolites. We wish to record the finding of a methylated oligoketide which may be a prototype of enzyme-bound higher polyketides.

In continuation of a program[11,12] involving characterization of the metabolites elaborated by a strain (NRRL 1006) of *Penicillium stipitatum*, several new compounds were isolated following chromatography on activated alumina of ethereal extracts from the fermentation beer.[13] One of these (**1a**) [mp 212–214°; λ_{max}^{EtOH} 288 mμ (ϵ 8300), unchanged by acid or base; λ_{max}^{KBr} 3.72, 5.99, 6.08, 6.31 μ; $[\alpha]D$ 0; pK = 5.05; neut equiv 139; nmr ($CDCl_3(CD_3)_2SO$) τ = 4.05, 5.06, 7.85, and 8.25 ppm, relative intensity 1:1:3:3[14] (*Anal.* Calcd for $C_7H_8O_3$: C, 59.99; H, 5.75. Found: C, 59.91; H, 5.79)] appeared to be closely related in structure to triacetic lactone (**1g**) or to dehydroacetic acid (**1f**), and for the foregoing biogenetic considerations was chosen for further characterization.

In order to demonstrate that **1a** was not an artifact resulting from the isolation procedure, control chromatograms were run using identical developing solvents with activated alumina columns. In these experiments, the presence of **1a** was not detected. To preclude the possibility that **1a** arose from facile rearrangement of a closely related metabolite on activated alumina, the following method for its isolation was developed in parallel. Crude stipitatic acid and its tropolone congeners were obtained from the ether extract of the fermentation beer. After crystallization from methanol, the filtrates were concentrated and



Ia, X = O; R^1 = H; R^2 = CH_3
b, X = O; R^1 = CH_3; R^2 = CH_3
c, X = O; R^1 = $COCH_3$; R^2 = CH_3
d, X = N–CH_3; R^1 = H; R^2 = CH_3
e, X = NH; R^1 = H; R^2 = CH_3
f, X = O; R^1 = H; R^2 = $COCH_3$
g, X = O; R^1 = H; R^2 = H
h, X = O; R^1 = $SO_2C_6H_4CH_3$-*p*; R^2 = CH_3
i, X = O; R^1 = $SO_2C_6H_4Br$-*p*; R^2 = CH_3

streaked across the origin of Whatman 3MM filter paper. Development of the paper chromatograms[15] showed the presence of **1a** in an ultraviolet absorbing band at R_f 0.6–0.9,[16] whereas residual tropolones were retarded with R_f's of around 0.2. Pure **1a** was obtained by elution from the dried paper with acidified ethanol, concentration of the alcoholic extract *in vacuo*, and sublimation of the residue. Samples of **1a** isolated in this manner, those obtained by partition chromatography on alumina, as well as the synthetic material (*vide infra*), had identical melting points and spectral properties.

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(13) Tabulation of these metabolites has been presented elsewhere: S. W. Tanenbaum, "Biogenesis of Antibiotic Substances," Z. Vanek and Z. Hostalek, Ed., Academic Press Inc., New York, N. Y., 1965, p 143.
(14) We are indebted to Dr. D. P. Hollis, Varian Associates, for nmr spectra obtained at 60 Mc using tetramethylsilane = 10 as reference.
(15) The developing solvent was the organic phase of the mixture chloroform–methanol–4% aqueous formic acid, 10:1:1 (v/v); one of the media described by L. Reio, *J. Chromatog.*, **1**, 338 (1958), for the separation of triacetic lactone and related compounds.
(16) The R_f of synthetic **1a** is 0.81 in this chromatographic system.

In order to gain an insight into the arrangement of the carbon skeleton of **1a**, it was first determined that prolonged refluxing in 2 *N* sodium hydroxide or in 2 *N* hydrochloric acid did not change the properties of this material; and, indeed, it could be recovered in quantitative yield. Heating of **1a** in quinoline with copper chromite catalyst caused no decarboxylation. Reduction of **1a** with phosphorus and iodine in glacial acetic acid gave an oil with a fatty acid like odor, which could not be crystallized, and which migrated on paper as a single spot with an R_f close to known C_6 aliphatic acids. In the presence of prehydrogenated Adams catalyst in glacial acetic acid, 3 moles of hydrogen was absorbed, and the infrared spectrum of this reaction mixture indicated bands corresponding to hydroxyl, methylene, and carboxyl groups. Paper chromatography indicated a mixture of C_4, C_5, and C_7 aliphatic acids to be present. Reduction with 5% rhodium on alumina in either glacial acetic acid or ethanol resulted in the uptake of 1 mole of hydrogen, and from these experiments "monoreduced" **1a**, mp 143–145°, could be isolated easily. This material (λ_{max}^{EtOH} 248 mμ, λ_{max}^{NaOH} 280 mμ; $pK = 5.36$) had an infrared spectrum which indicated the presence of an associated hydroxyl and a carbonyl in conjugation with a double bond. It has never given satisfactory analyses for the expected $C_7H_{10}O_3$ (*Anal.* Calcd: C, 59.14; H, 7.09. Found: C, 62.80; H, 7.64) and determination of its structure has not been pursued further. However, "monoreduced" **1a**, either on heating or prolonged standing with 0.1 *N* sulfuric acid or upon dry distillation (oil bath at 150°, nitrogen stream), liberated carbon dioxide. These experiments indicated that **1a** contained the potential structural elements of a β-keto acid. The supposition that this masked ketone was probably present as a stable enol was indicated further by the preparation of crystalline tosyl, brosyl, acetyl, and methyl derivatives of **1a**. Attempts to elucidate the carbon skeleton by Raney nickel reduction of **1h** or of **1i** were inconclusive.

When **1a** was treated with methylamine there was obtained a nitrogen-containing derivative ($C_8H_{11}NO_2$) in which one oxygen appeared to have been replaced by NCH_3. This compound (**1d**) had an absorption maximum at 288 mμ which shifted to 279 mμ in the presence of base. Based upon the work of Berson, *et al.*,[17] these chemical and spectral properties were indicative of the 4-hydroxy-2-pyrone system and its corresponding N-methylpyridone. Further provisional identification of **1a** was accomplished by its reaction with ammonia. The anticipated pyridone (**1e**), mp 268–270°, was identical in all aspects with authentic 3,6-dimethyl-4-hydroxy-2-pyridone which had been described previously.[18] It may be of some interest to note that the isomeric 3,5-dimethyl-4-hydroxy-2-pyrone has been obtained from the pyrolysis of 1-formylpropionate,[19] but that the 5,6-dimethyl isomer of the natural product is as yet unknown.

While **1a** did not react with methanol–sulfuric acid, its treatment with either diazomethane or dimethyl sulfate gave the methyl derivative (**1b**), λ_{max}^{EtOH} 297 mμ. The 4-methoxy structure is assigned to this compound on the basis of this ultraviolet absorption maximum.[20] Reaction of **1a** with acetic anhydride gave the acetyl derivative (**1c**), which was neutral but produced two acidic groups under mild basic conditions. It was assigned the 4-acetoxy structure on the basis of this fact and upon its absorption maximum at 293 mμ.[17]

Examination of the literature indicated that the synthesis of **1a** indeed had been reported,[21] but without experimental details. This work was based on the previously outlined general method[22] for the preparation of 4-hydroxy-2-pyrones which involved the low-yield condensation of ethyl acetoacetate with malonyl chloride derivatives. At the outset, it appeared more expedient to attempt the preparation of **1a** from the readily available triacetic lactone (**1g**) by halomethylation followed by selective dehydrohalogenation, since it was anticipated that electrophilic substitution would occur on the appropriate carbon of this ring system. However, it was not possible to isolate the halomethyl derivative. Under a variety of experimental conditions only the earlier described[23] methylene dimer of triacetic lactone was obtained. Consequently, **1a** was synthesized as previously outlined[21] using initial condensation of acetoacetate and methylmalonic acid, no advantage accruing from use of the acid chloride. From the intermediary 5-carbethoxy-3,6-dimethyl-4-hydroxy-2-pyrone, there was obtained on hydrolysis the corresponding carboxylic acid, which upon decarboxylation gave the desired compound. For the synthesis of radioactive **1a**, alkylation of malonic ester-2-C^{14} was carried out by the method of Zuagg, *et al.*,[24] to minimize dialkylation. After saponification of the labeled methylmalonic ester, condensation and subsequent steps were carried out as given above. By this procedure, starting with malonic ester-2-C^{14} (specific radioactivity 1 mcurie/mmole) and after subsequent additions of carrier, there was obtained 5.4 mmoles of 3,6-dimethyl-4-hydroxy-2-pyrone-3-C^{14} (specific radioactivity 23.4 μcuries/mmole).

The biogenesis of **1a** can be hypothesized to occur either from an acetate unit being condensed sequentially with malonyl CoA and methylmalonyl CoA; or from 1 mole of "starter" acetyl CoA and 2 moles of malonyl CoA followed by biological methylation, the latter step being analogous to the formylation of metal acetylacetonates.[25] In either case, the isolation of **1a** suggests that many of the "extra" methyl groups, unaccountable in terms of the straightforward acetate–polymalonate route, and which occur in a variety of fungal products,[5] are attached to polyketide intermediates prior to their cyclization. The fact that **1a** is found in the metabolism filtrate of *P. stipitatum* prior to the appearance of tropolone acids implies, if it is at all involved with the biosynthesis of these substances,

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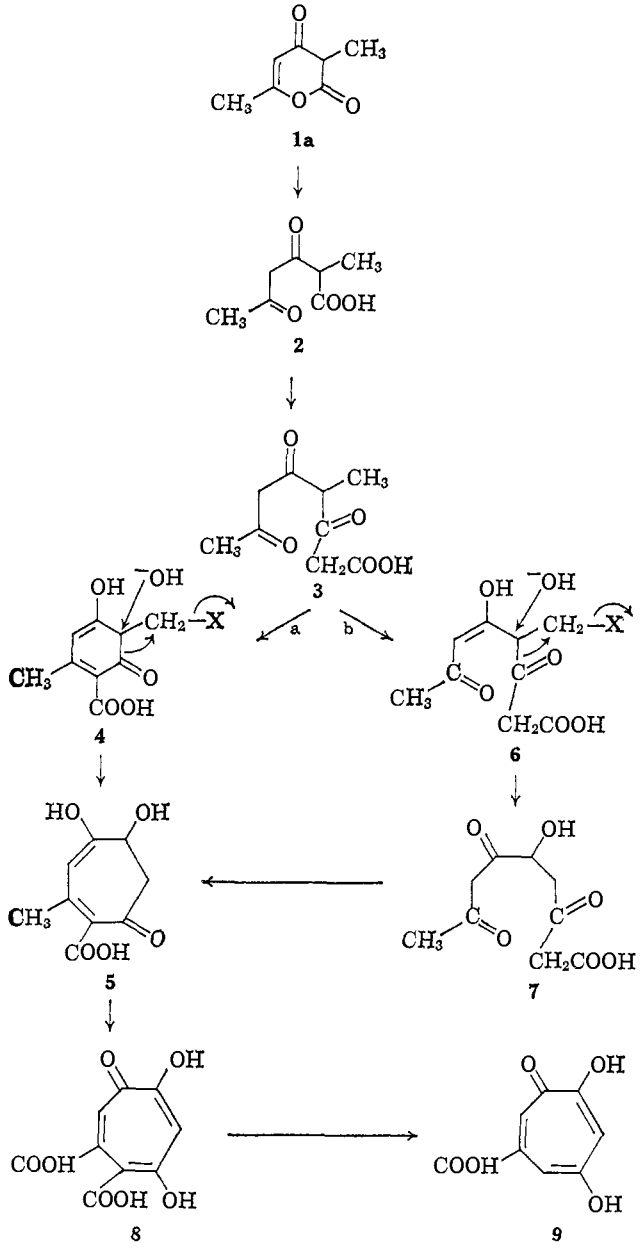
that it might be a precursor rather than a degradation product. One possible metabolic sequence to stipitatic acid involving **1a** can be envisioned as given in Scheme I. In this series of reactions, **1a**, in hydrated open-chain structure, 2-methyl-3,5-dioxohexanoic acid (**2**), is postulated to undergo further chain elongation

Scheme I



with malonyl CoA to give the next higher polyketide (**3**). This intermediate in turn can be assumed, while attached to a multienzyme complex, to be involved in a series of reactions (pathways a or b)[26] to give **5** or **7**, hypothetical cyclic or open-chain precursors of the tropolone acids. This postulated ring expansion (**4** to **5**) is reminiscent of laboratory procedures known to produce troponoid systems from solvolytic reactions of dihydrobenzyl compounds,[27,28] and by reactions of 2-methylbenzoquinol 2-acetates[29] with diazomethane. A perhaps more attractive biological mechanism for the conversion of **4** (or of **6**) to **5** might be by methyl insertion, analogous to the transformation of methylmalonic to succinic acid,[30] followed by hydroxylation. Although the commonly occurring mold metabolite orsellinic acid has been alternatively suggested as an immediate precursor of the tropolone ring system, the most recent experimental evidence[31] still leaves its role in this process doubtful, and this intermediate has been omitted from the scheme. The participation of **1a** in stipitatic acid biogenesis would still result in the same isotope distribution pattern previously obtained[26,32,33] from incorporation studies which used labeled acetate and formate. With the availability of radiochemically labeled species of **1a**, its presumptive role in fungal tropolone biosynthesis can be further examined.

Experimental Section[34]

Isolation of 3,6-Dimethyl-4-hydroxy-2-pyrone (1a). *Penicillium stipitatum* (NRRL 1006) was grown in stationary culture at 37° in 2.8-l. fernbach flasks on 500 ml of 5% glucose Czapek-Dox medium, in which the ferrous sulfate was replaced by 1 ml/l. of a special inorganic salt solution.[12] After 14 days of growth, the mycelium was removed by filtration, and the filtrate was concentrated under vacuum to approximately 0.5% of its original volume. The dark brown solution was acidified to congo red with 6 *N* hydrochloric acid and cooled. The precipitated crude stipitatic acid was removed, and the filtrate was extracted continuously with ether. The crude stipitatic acid was recrystallized twice from methanol. The methanol mother liquors and the ether extract were combined, and were brought to dryness by flash evaporation. The gummy residue was taken up in ethanol–ethyl acetate (1:1) and chromatographed on a column of neutral alumina (Woelm activity grade 1). The column was developed sequentially with the following solvents: benzene, benzene–ethyl acetate (1:1), ethyl acetate, ethyl acetate–acetone (1:1), acetone, acetone–ethanol (4:1), acetone–ethanol (2:1), and acetone–ethanol (1:1). The residue remaining after evaporation of the acetone–ethanol (4:1) eluate was sublimed at 120° (0.3 mm). The sublimate was recrystallized from acetone–petroleum ether (bp 30–60°) and gave 3,6-dimethyl-4-hydroxy-2-pyrone; yield 45 mg/l.; mp 212–214° (lit.[21] mp 208–209°); λ_{max}^{EtOH} 288 mμ (ε 8300), unchanged on the addition of base and acid; λ_{max}^{KBr} 3.72, 5.99, 6.08, 6.31 μ; $[\alpha]D$ 0; p*K* = 5.05; neut equiv 139; nmr [$CDCl_3(CD_3)_2SO$) τ = 4.05, 5.06, 7.85, and 8.25 ppm, relative intensity 1:1:3:3.

Anal. Calcd for $C_7H_8O_3$: C, 59.99; H, 5.75. Found: C, 59.91; H, 5.79.

Comparable alumina chromatograms which used 4-day cultures of *P. stipitatum* indicated the presence of *ca.* 6 mg/l. of **1a**, whereas stipitatic acid did not make its appearance until the seventh day of growth.

Reduction of 1a with 5% Rhodium Catalyst. A solution of 210 mg of **1a** (1.5 mmoles) dissolved in 25 ml of glacial acetic acid was reduced at atmospheric pressure using 100 mg of prehydrogenated 5% rhodium-on-alumina catalyst. After 5 min, 1 mole equiv of hydrogen was taken up, and gas uptake ceased. The mixture was filtered, the solvent was removed under reduced pressure at low temperature, and colorless crystals (mp 110–130°) remained. This material was crystallized from acetone–petroleum ether to give white needles; mp 141–143°, λ_{max}^{EtOH} 248 mμ, λ_{max}^{NaOH} 280 mμ. "Monoreduced" **1a** reacted with Brady's solution to form a mixture of 2,4-dinitrophenylhydrazones, and was easily decarboxylated by heat or with 0.1 *N* sulfuric acid.

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(34) All radioactivity measurements were made by Van Slyke wet combustion of samples to barium carbonate, followed by replicate plating at infinite thinness. Counts were determined using an end window counter with *ca.* 7% efficiency.

3,6-Dimethyl-4-methoxy-2-pyrone (1b). To 10 ml of acetone, dried over potassium carbonate and distilled from potassium permanganate, was added 27 mg (0.2 mmole) of **1a**, 0.3 ml of dimethyl sulfate, and 0.3 g of anhydrous potassium carbonate, and the mixture was refluxed for 84 hr. The acetone solution was filtered and the acetone was evaporated. The white solid residue was sublimed at 110° (0.07 mm) to give 14 mg (47% yield) of 3,6-dimethyl-4-methoxy-2-pyrone, mp 82–83° (lit.[21] mp 86–87°).

Anal. Calcd for $C_8H_{10}O_3$: C, 62.32; H, 6.54. Found: C, 62.44; H, 6.58.

4-Acetoxy-3,6-dimethyl-2-pyrone (1c). A solution of 35 mg (0.25 mmole) of **1a**, 2 ml of acetic anhydride, and 2 ml of pyridine was maintained at 37° for 7 days. The residue that remained after evacuation with a mechanical oil pump was crystallized from petroleum ether–acetone to give 40 mg of 4-acetoxy-3,6-dimethyl-2-pyrone, mp 84–85°.

Anal. Calcd for $C_9H_{10}O_4$: C, 59.33; H, 5.53. Found: C, 59.52; H, 5.63.

The acetyl compound was neutral but on standing for 3 hr in dilute alkali consumed 2 moles of base.

3,6-Dimethyl-4-*p*-toluenesulfoxy-2-pyrone (1h). A solution of 35 mg (0.25 mmole) of **1a**, 52 mg of *p*-toluenesulfonyl chloride, and 2 ml of absolute pyridine was maintained at 37° for 6 days. After evacuation at 0.3 mm, the oily residue was treated with water. The white solid was filtered and crystallized from acetone–petroleum ether to give the tosyl derivative, mp 113–115°.

3,6-Dimethyl-4-(4-bromobenzenesulfoxy)-2-pyrone (1i). A solution of 70 mg (0.5 mmole) of **1a**, 30 mg of 4-bromobenzenesulfonyl chloride, and 2 ml of absolute pyridine was maintained at 37° for 6 days. After evacuation at 0.3 mm, the residue was treated with water containing a few drops of hydrochloric acid; the white solid was filtered off and was then dried to give 102 mg (57% yield) of 3,6-dimethyl-4-(4-bromobenzenesulfoxy)-2-pyrone. This product was crystallized from acetone–ether, mp 161–162°.

Anal. Calcd for $C_{13}H_{11}BrO_5S$: Br, 22.25. Found: Br, 22.20.

4-Hydroxy-1,3,6-trimethyl-2-pyridone (1d). A solution of 109 mg (0.78 mmole) of **1a** in 10 ml of 25% aqueous methylamine was heated overnight in a water bath at 85°. The solvent was removed at water-pump pressure and the residue was rinsed with water, dried, and sublimed at 120° (0.025 mm) to give 70 mg (59% yield) of 4-hydroxy-1,3,6-trimethyl-2-pyridone as white needles from water: mp 265°, λ_{max}^{EtOH} 288 mμ, λ_{max}^{NaOH} 279 mμ.

Anal. Calcd for $C_8H_{11}NO_2$: C, 62.72; H, 7.24; N, 9.15. Found: C, 62.99; H, 7.37; N, 9.00.

2,4-Dihydroxy-3,6-dimethylpyridine (1e). A solution of 9 mg (0.064 mmole) of **1a** in 0.5 ml of concentrated aqueous ammonia was heated in a sealed tube at 120° for 6 hr, allowed to stand at room temperature overnight, and brought to dryness at water-pump pressure. The residue was rinsed with water, dried, and sublimed at 160° (0.03 mm) to give 4 mg (50% yield) of 2,4-dihydroxy-3,6-dimethylpyridine: mp 268–269° (lit.[18] mp 276°), λ_{max}^{EtOH} 288 mμ, λ_{max}^{NaOH} 278 mμ, λ_{max}^{KBr} 6.05 μ, identical by melting point, mixture melting point, and superimposable infrared spectrum with authentic material.

Di-3,3′-(4-hydroxy-6-methyl-2-pyrone)methane. (A). To 448 mg (3.5 mmoles) of triacetic lactone in 17 ml of acetic acid was added a solution of 1.15 g of paraformaldehyde in 9 ml of 47% hydriodic acid. The resulting solution was allowed to stand overnight. The precipitate was filtered, rinsed with ether, and dried under vacuum to give 149 mg of a yellow solid. All attempts at purification led to decomposition. A mixture of this material dissolved in 10 ml of 5% sodium bicarbonate with 26 mg of 5% palladium on calcium carbonate was stirred under hydrogen for 3 hr. The solution was filtered through Celite and acidified with concentrated hydrochloric acid. From this was isolated 56 mg of a white solid, mp 230–235°. The melting point was raised to 251–253° after recrystallization from methanol. This preparation had an infrared spectrum identical with authentic material synthesized as given below.

Anal. Calcd for $C_{13}H_{12}O_6$: C, 59.09; H, 4.58. Found: C, 59.12; H, 4.61.

(B). To 204 mg of 4-hydroxy-6-methyl-2-pyrone (1 g) in 2 ml of methanol was added 1 ml of formalin. A white precipitate formed rapidly. It was removed, dried, and crystallized from 95% ethanol, and there was obtained 180 mg of di-3,3′-(4-hydroxy-6-methyl-2-pyrone)methane, mp 251–253° (lit.[23] mp 245°).

5-Carbethoxy-3,6-dimethyl-4-hydroxy-2-pyrone. To 5 ml of benzene, dried by azeotropic distillation, was added 1.18 g (0.01 mole) of methylmalonic acid, 1.30 g (0.01 mole) of ethyl acetoacetate, and 1.5 ml of thionyl chloride. The mixture was heated at 90° for 18 hr, cooled, evacuated for a short while with the water pump, and then extracted with sodium bicarbonate solution. The basic solution was acidified with hydrochloric acid. The precipitated dark brown oil was extracted into ether. After ether removal, the residue was distilled through a short-path apparatus to give 0.2 g (10% yield) of 5-carbethoxy-3,6-dimethyl-4-hydroxy-2-pyrone, bp 118° (0.25 mm). This material was used directly for further transformations. For analysis, 30 mg was chromatographed with benzene on 623 mg of neutral alumina (Woelm activity grade 4). The residue obtained after removal of the solvent was sublimed at 85° (0.2 mm) to give 21 mg of pure compound: mp 53–54°; λ_{max}^{EtOH} 263, 270 mμ; λ_{max}^{KBr} 5.8, 5.9, 6.1, 6.4 μ.

Anal. Calcd for $C_{10}H_{12}O_5$: C, 56.60; H, 5.70. Found: C, 56.40; H, 5.61.

5-Carboxy-3,6-dimethyl-4-hydroxy-2-pyrone. A mixture of 2.5 g (11.8 mmoles) of 5-carbethoxy-3,6-dimethyl-4-hydroxy-2-pyrone, 5.9 g of barium hydroxide hydrate, and 50 ml of water was heated together on the steam bath for 2 hr and then was allowed to stand for 1 hr. After chilling, the reaction mixture was acidified with concentrated hydrochloric acid and was kept in the refrigerator overnight. The insoluble material was filtered and air dried to give 1.18 g (54% yield) of 5-carboxy-3,6-dimethyl-4-hydroxy-2-pyrone, mp 246–248° dec. This was used directly for the next step. For analysis it was recrystallized from acetone to give material, mp 251–252°.

Anal. Calcd for $C_8H_8O_5$: C, 52.18; H, 4.38. Found: C, 51.88; H, 4.59.

3,6-Dimethyl-4-hydroxy-2-pyrone (1a). To 17 ml of nitrobenzene containing 3 drops of quinoline was added 2.0 g (10.8 mmoles) of 5-carboxy-3,6-dimethyl-4-hydroxy-2-pyrone. The mixture was maintained at 210° for 15 min and then cooled in ice. The precipitated solid was filtered, washed with ether, and dried under reduced pressure on the steam bath and then under high vacuum at room temperature to give 1.1 g (72% yield) of 3,6-dimethyl-4-hydroxy-2-pyrone, mp 208–209°. From a sample of 103 mg of this material, 94 mg was soluble in 95% ethanol, and this upon crystallization from 5 ml of water yielded 82 mg of crystalline material, mp 209°. The synthetic compound was identical by mixture melting point and infrared spectrum with the natural substance.

3,6-Dimethyl-4-hydroxy-2-pyrone-3-C^{14}. To 13 ml of benzene, dried by azeotropic distillation, was added 684 mg of 85% potassium hydroxide (10.4 mmoles), 160 mg of diethyl malonate-2-C^{14} (1 mcurie/mmole), and 1.62 g of carrier ester. The mixture was heated in a bath at 90° for 1 day using a Dean–Stark apparatus which contained Linde molecular sieve 4A. The solvent was removed at reduced pressure and room temperature. The residual salt was dissolved in 7 ml of dimethylacetamide and to this was added 1.74 g (12.2 mmoles) of methyl iodide in 1 ml of dimethylacetamide.[24] The resulting mixture was maintained at 100° for 0.5 hr.

The labeled ethyl methylmalonate was diluted with 339 mg of carrier and was treated with 2.5 g (62.5 mmoles) of sodium hydroxide in 6 ml of water and 2 ml of dimethyl sulfoxide. The solution was cooled in tap water and stirred for 0.5 hr. After addition of ether and water, the basic aqueous solution was separated, washed twice with ether, and acidified with hydrochloric acid. It was continuously extracted with ether for 1 day. The ether solution was washed with saturated sodium chloride and dried over sodium sulfate, and the ether was removed at room temperature under vacuum. The residue was washed with *n*-hexane to give 650 mg (55% yield) of methylmalonic acid, mp 120–126°. This was diluted to 1.30 g with carrier to give material of specific radioactivity, 26 μcuries/mmole.

5-Carbethoxy-3,6-dimethyl-4-hydroxy-2-pyrone-3-C^{14} was obtained from the diluted methylmalonic acid-2-C^{14}, in 14% yield by the procedure described above. Its specific activity was determined to be 28 μcuries/mmole. From 334 mg of this ester, 154 mg (53% yield) of 5-carboxy-3,6-dimethyl-4-hydroxy-2-pyrone-3-C^{14} (specific radioactivity, 21 μcuries/mmole) was obtained by saponification. After decarboxylation, the reaction mixture was evacuated (0.2 mm) at room temperature to give 124 mg of a dark brown residue, which after sublimation (130–135° at 0.05 mm) and water crystallization provided 76 mg (65% yield) of 3,6-dimethyl-4-hydroxy-2-pyrone-3-C^{14} (specific radioactivity, 23.4 μcuries/mmole).