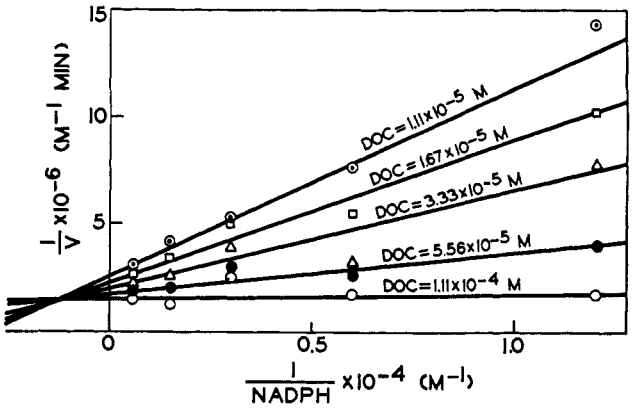

Figure 1. Double-reciprocal plots of $1/V$ *vs.* 1/[NADPH] at several fixed concentrations of DOC. The system consists of 5 mg of P_2 fraction (P_{450}), 0.2 mg of R_1 fraction (NHIP), 0.2 mg of Y_2 fraction (FP) (see ref 12 for details of fractionation), and 5 μmol of $MgCl_2$, in a total volume of 3 ml of 0.1 *M* phosphate buffer, pH 7.5. The reaction mixture was incubated for 10 min at 37°. Corticosterone was assayed by a radiochromatographic method.

The proposed mechanism avoids the cogent objection that a ferrous ion–oxygen complex should not be reactive enough to enter directly into oxidations such as those of unactivated carbon–hydrogen bonds. It seems reasonable that a much more highly reactive species is required for this. Postulation of reduction of an iron–oxygen complex with formation of a reactive species is consistent with studies of the autoxidation of Fe^{2+}, whose second-order dependence on ferrous ion concentration suggests rate-determining reduction of a ferrous ion–oxygen complex.[21,22] It is likely that proposed scheme is of general physiological significance, common to all external mixed-function oxidases.[23]

Acknowledgment. The authors are indebted to Mr. Michael Lemberger for the large-scale preparation of adrenal cortex mitochondria.

(21) R. E. Huffman and N. Davidson, *J. Am. Chem. Soc.*, 78, 4836 (1956).
(22) P. George, *J. Chem. Soc.*, 4349 (1954).
(23) This investigation was supported by research grants from the National Institutes of Health (AM-4874 and AM-6110).

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Biogenetic Relationship between Methyl Triacetic Lactone and Stipitatic Acid

Sir:

Recent isolations of methyl triacetic lactone[1] (3,6-dimethyl-4-hydroxy-2-pyrone, **1**), triacetic lactone,[2,3] and tetraacetic lactone[2] from higher fungi which simultaneously produce tropolones or phenols have provoked speculation[1-3] on the role of mutual progenitor poly-β-ketides[4] in the formation of these metabolites. We wish to report data which demonstrate that the extended, presumably enzyme-bound, acetate–polymalonate precursor to **1** is probably involved in the formation of stipitatic acid (**5**), and that the origin of the augmented methyl or methyl-derived carbons in *both* metabolites appears to arise from the same single-carbon transfer pool. After growth in the presence of sodium formate-^{14}C, cultures of *Penicillium stipitatum* NRRL 1006 afforded samples of **1** and **5** which were isolated and degraded as outlined below. Intermediates in the stepwise degradation of **1** (Figure 1A) were shown to have uv, ir, and mass spectra and elemental analyses consonant with their structures; the ultimate 3-methyl-5-pyrazolecarboxylic acid (**4**) ex-

(1) (a) P. E. Brenneisen, T. E. Acker, and S. W. Tanenbaum, *J. Am. Chem. Soc.*, **86**, 1264 (1964); (b) T. E. Acker, P. E. Brenneisen, and S. W. Tanenbaum, *ibid.*, **88**, 834 (1966).
(2) R. Bentley and P. M. Zwitkowitz, *ibid.*, **89**, 676, 681 (1967).
(3) (a) T. M. Harris, C. M. Harris, and R. J. Light, *Biochim. Biophys. Acta*, **121**, 420 (1966); (b) R. J. Light, T. M. Harris, and C. M. Harris, *Biochemistry*, **5**, 4037 (1966).
(4) A. J. Birch, *Science*, **156**, 202 (1967).

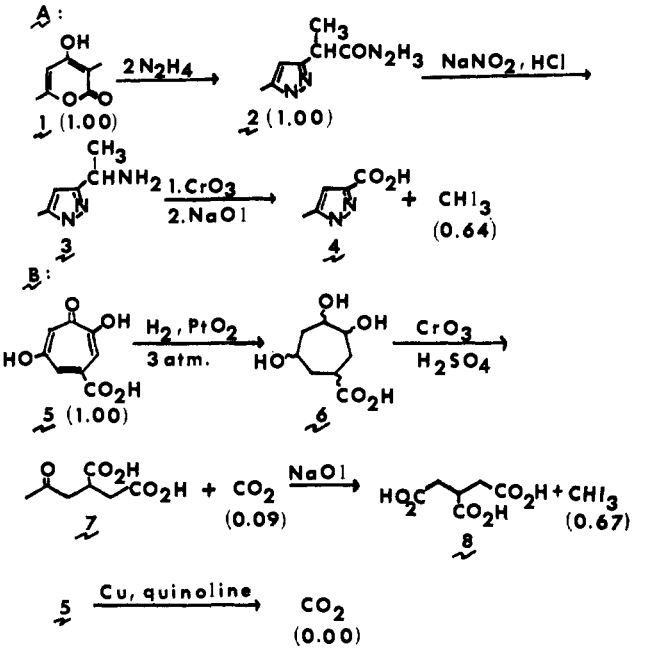

Figure 1. Partial degradations of methyl triacetic lactone and of stipitatic acid.

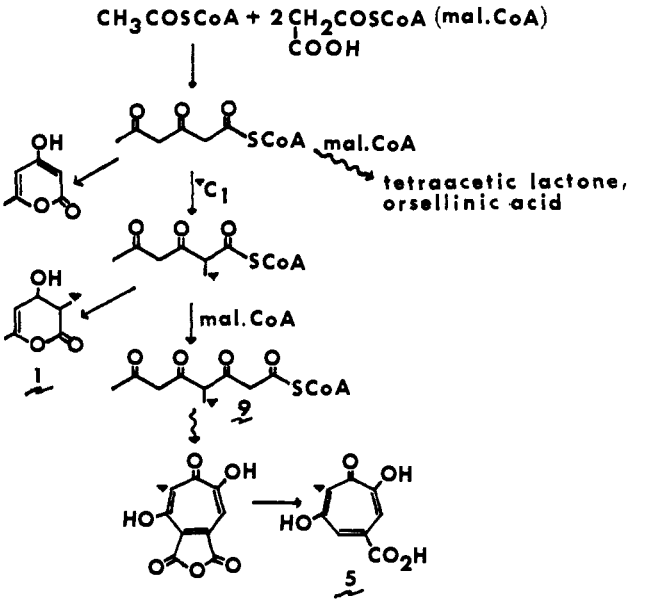

Figure 2. Proposed biosynthetic interrelationships among fungal polyketides and their metabolic congeners. In actuality other 4′-phosphopantetheine carriers, such as ACP, may be involved in oligoketide transfers.

Table I. Utilization of Radiolabeled Formate and Dimethyl Methylmalonate by *P. stipitatum*[a]

Compd	$H^{14}COOH$ Yield, g	$H^{14}COOH$ Spec. act., dpm/mmol × 10^{-7}	$H^{14}COOH$ Incorpn, %	$^{14}CH_3CH(COOCH_3)_2$ Yield, g	$^{14}CH_3CH(COOCH_3)_2$ Spec act., dpm/mmol × 10^{-5}	$^{14}CH_3CH(COOCH_3)_2$ Incorpn, %
Methyltriacetic lactone	0.040	1.45	1.6	0.035	1.79	0.02
Stipitatic acid	1.20	1.61	41	1.10	0.745	0.21
Iodoform from **1**		0.93[b,c]				
Iodoform from **5**		1.08[b]				

[a] Cultures were grown in 500 ml of medium at 37° for 5 days,[1] after which time radioactive supplements (formate 117 μCi, specific activity 3.0 mCi/mmol; methylmalonate, 100 μCi, specific activity 1.9 mCi/mmol) were added. After 6 additional days, metabolites (yields expressed per fermentation flask) were isolated by silica gel chromatography of concentrated ethereal extracts. They were purified to constant melting point, chromatographic homogeneity, and radioactivity by repeated crystallization and sublimation. [b] Calculated from values obtained after appropriate dilutions with nonisotopic carriers. Actual activities of iodoform species from **1** and **5** were 480 dpm/mg and 1332 dpm/mg, respectively, in a liquid scintillation system counting at 84% efficiency. Appropriate quenching corrections have been applied to each sample counted. [c] Microscale isolation procedures and a facile synthesis of carrier **1** will be described in a forthcoming publication.

hibited no depression in melting point with a sample prepared from triacetic lactone.[5] Although the intermediates in the novel degradation scheme for **5** (Figure 1B) have not as yet been individually characterized, the validity of this pathway was assured not only by obtaining CO_2 and CHI_3 at anticipated stages but also by identification of the resultant tricarballylic acid (**8**) by comparison of the retention times of its trimethyl ester on two vpc columns with those for the authentic derivative.

The close specific molar radioactivities of **1** and **5**, as well as the almost identical activities within those selected carbons from each metabolite to which formate made the major contribution (Table I), strongly suggest a biogenetic kinship. The relationship of the immediate polyketide precursor to both **1** and **5** is all the more striking in light of the fact that the former metabolite is some 30-fold less concentrated in the fermentation beer than the latter. Additional relative molar distributions of radioactivity which were determined for **5** are shown in Figure 1. These data for incorporation of radioactive formate into **5** qualitatively accord with earlier studies[6,7] on tropolone biosynthesis which employed less straightforward methods of degradation for obtaining C_7. The relatively poor incorporation of initial radioactivity from dimethyl methyl-^{14}C-malonate into **1** (Table I) would seem to resolve the question[1] of whether the "extra" methyl group of this metabolite arises by a methylmalonyl chain-extension step or by C_1 transfer to a preformed polyketide.

Based on these results, proposed causal relationships among polyketide lactones, their precursors, and their phenolic congeners are given in Figure 2. We have modified earlier suggestions[1] in that the formation of triacetic lactone derivatives[8] are shown as side reactions subsequent to displacement of their linear, coenzyme-ligated polyketides from the aromatic multienzyme[9,10] complex. Indeed, the spontaneous cyclization of polyketo thiolesters has been noted.[3] Therefore, the addition of exogenous triacetic lactones to the growing mycelium results[2,3,11] in catabolism[12] rather than reversible ring opening with concomitant transfer to the appropriate anabolic enzyme surface. Details concerning the final stages of molecular rearrangement and cyclization to tropolones of the polyketide formally related in structure to the enzyme-bound 4-methyl-3,5,7-trioxooctanoyl moiety (**9**) can most likely only be ascertained by use of cell-free enzyme systems.

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(6) R. Bentley, *Biochim. Biophys. Acta*, **29**, 666 (1958).
(7) L. D. Ferretti and J. H. Richards, *Proc. Natl. Acad. Sci. U. S.*, **46**, 1438 (1960).
(8) No chromatographic evidence was found for the production of triacetic lactone or of tetraacetic lactone (authentic sample kindly provided by Dr. R. Bentley) by this particular isolate of *P. stipitatum* during growth under cultural conditions routinely employed[1] in this laboratory.
(9) F. Lynen and M. Tada, *Angew. Chem.*, **73**, 513 (1961).
(10) R. J. Light and L. P. Hager, *Arch. Biochem. Biophys.*, **125**, 326 (1968).
(11) Unpublished observations (T. E. Acker and S. W. Tanenbaum) have also shown that administration of biosynthetic radiolabeled **1** to noninhibited cultures of *P. stipitatum* 1006 resulted in minimal incorporation of tracer into **5**; the major radioactive mycelial constituent was ergosterol.
(12) R. F. Witter and E. Stotz, *J. Biol. Chem.*, **176**, 485, 501 (1948).
(13) Supported by a grant (AI-06801) from the U. S. Public Health Service.

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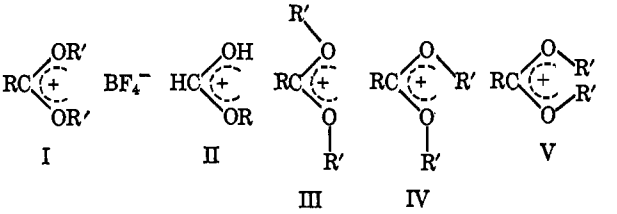
Alkoxycarbonium Ions. The Structure of O-Alkylated Esters

Sir:

As part of an investigation of strong alkylating agents for the preparation of nitrilium salts,[1] we have prepared a number of O-alkylated ester fluoroborates (I). In contrast to Olah's observations[2] on the protonated formates (II), the nmr study of these compounds indicates that the major isomer at low temperatures is the "*cis,trans*" isomer IV, not the "*cis,cis*" isomer III.



I II III IV V

a, R = H; R′ = CH_3
b, R = H; R′ = CH_2CH_3
c, R = H; R′ = $CH_2CH_2CH_3$
d, R = CH_3; R′ = CH_2CH_3

(1) R. F. Borch, submitted for publication.
(2) G. A. Olah, D. H. O'Brien, and A. M. White, *J. Am. Chem. Soc.*, **89**, 5694 (1967).