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.

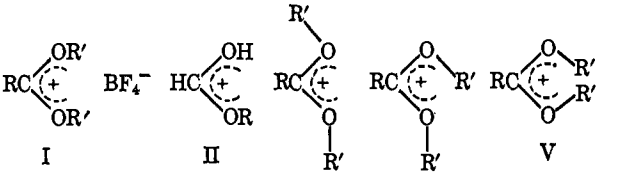
(5) C. Ainsworth and R. G. Jones, *J. Am. Chem. Soc.*, **76**, 3172 (1954).
(6) R. Bentley, *Biochim. Biophys. Acta*, **29**, 666 (1958).
(7) L. D. Ferretti and J. H. Richards, *Proc. Natl. Acad. Sci. U. S.*, **46**, 1438 (1960).
(8) No chromatographic evidence was found for the production of triacetic 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.

Gerard S. Marx, Stuart W. Tanenbaum[13]
Department of Microbiology, College of Physicians and Surgeons Columbia University, New York, New York 10032
Received July 9, 1968

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.

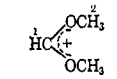
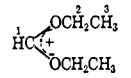
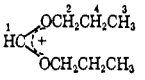
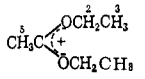


I II III IV V

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).

Table I. Nmr Chemical Shifts of O-Alkylated Ester Fluoroborates at +38° in $CDCl_3$[a]

O-Alkylated ester	δ, ppm from internal TMS				
	H_1	H_2	H_3	H_4	H_5
	−9.11 (s)[b]	−4.64 (s)			
	−9.09 (s)	−4.97 (q)	−1.59 (t)		
	−9.10 (s)	−4.92 (t)	−1.05 (t)	−1.99 (m)	
		−4.83 (q)	−1.51 (t)		−2.57 (s)

[a] Spectra were run on a Varian A-60 spectrometer equipped with a variable-temperature probe. [b] Obtained in CH_3NO_2–CH_2Cl_2 as solvent.

The esters I were prepared by a modification of Meerwein's procedure[3] by reaction of the corresponding orthoester and BF_3 at low temperature; the compounds were solids melting below 10° and, in contrast to the protonated esters,[2] were stable at room temperature. The O-alkylated esters gave well-resolved nmr spectra; the spectral data at +38° are summarized in Table I.

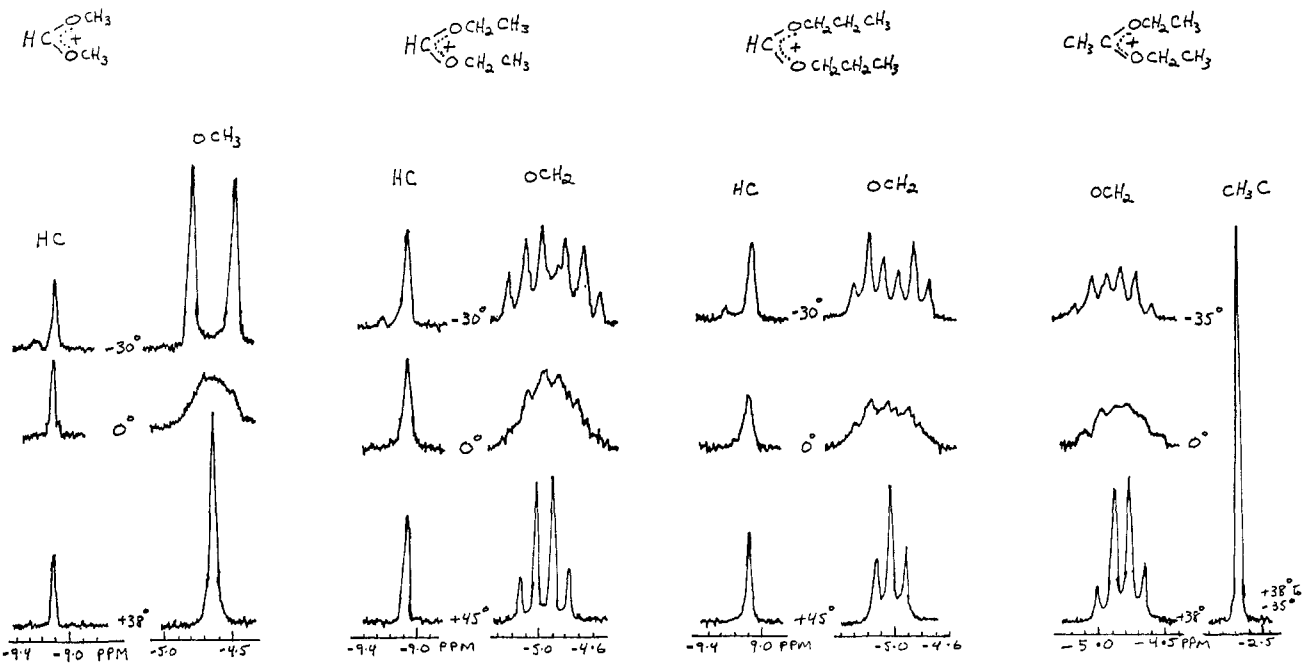


Figure 1.

The formate hydrogens appear as singlets (width at half-height 2.5 Hz) at −9.1 ppm; the remaining hydrogens are shifted downfield from the corresponding hydrogens in the simple esters and can be readily assigned on the basis of their multiplicities.

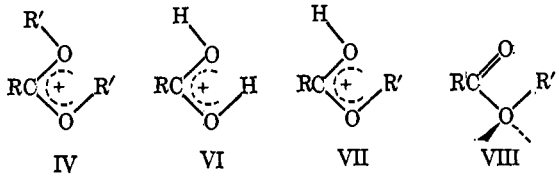
At +38° the two O-alkyl groups in each compound are equivalent and give one sharp set of lines. As the temperature is decreased, however, the OC–H proton signals are broadened (see Figure 1); at −30° these protons appear as two sets of lines approximately equal in area, separated by 0.32 ppm in the formates and by 0.22 ppm in the acetate. The H–C proton in the formates and the CH_3–C protons in the acetate remain as essentially one peak. The acetate is exclusively one isomer; a small peak observed 0.1–0.2 ppm downfield from the H–C peak in the formates indicates the presence of ~10% of another isomer.

We interpret these spectral changes as being a result of the predominance (>90%) of one isomer at −30° in which the O-alkyl groups are nonequivalent, *i.e.*, isomer IV; since Olah did not observe any isomer V in either the protonated acids[4] or esters,[2] the minor isomer is presumably III. We cannot reconcile our data with the assignment of III as the predominant isomer, as predicted from Olah's assignments for protonated ethyl formate.[2] Because his assignments were made only on the basis of chemical shift differences,[2] his data can be reconciled with IV as the predominant isomer for protonated ethyl formate and for the other protonated esters which he examined.

(3) H. Meerwein, K. Bodenbenner, P. Borner, F. Kunert, and K. Wunderlich, *Ann.*, **632**, 38 (1960).

(4) G. A. Olah and A. M. White, *J. Am. Chem. Soc.*, **89**, 3591 (1967).

There emerges from this interpretation of the nmr data a pattern of consistency for the protonated acids VI,[4] the protonated esters VII, and the O-alkylated esters IV. For the formates (VI, VII, and IV where R = H; R′ = alkyl) the "*cis,trans*" form predominates to the extent of 70–90%, and for the acetates and higher homologs (VI, VII, and IV where R and R′ = alkyl) the "*cis,trans*" form is the exclusive isomer. This result is not unexpected in view of the known[5] conformational preference of aliphatic esters (VIII), where the alkyl group is coplanar and "*cis*" to the carbonyl oxygen.



IV VI VII VIII

Acknowledgment. The author is indebted to the Petroleum Research Fund (No. 710-G), administered by the American Chemical Society, for financial support, and to R. M. Dodson for helpful discussions during the course of this work.

(5) J. E. Piercy and S. V. Subrahmanyan, *J. Chem. Phys.*, **42**, 1475 (1965), and references cited therein.

Richard F. Borch
Department of Chemistry, University of Minnesota
Minneapolis, Minnesota 55455
Received July 1, 1968

Diastereoisomeric Four-Coordinate Complexes. VII.[1] Proton Resonance Detection of the Nonequivalence of Enantiomeric Nickel(II) Complexes in an Optically Active Solvent

Sir:

Recent reports have dealt with the pmr spectra of enantiomeric mixtures of organic compounds dissolved in optically active solvents and recorded in either liquid[2] or nematic phases.[3] Observations in the liquid phase are of considerable importance because in favorable instances of large chemical shift separations between signals of enantiomers, produced by diastereoisomeric solute–solvent interactions, assessment of optical purities[2a] and assignment of absolute configurations[2d] of solutes have been possible. We report here the first instance of diastereoisomeric interactions between a dissymmetric metal complex and an optically active solvent observable by proton resonance.

The quadridentate nickel(II) complexes Ni(3*s*Bu,5X′-sal)$_2$B (*cf.* Figure 1), derived from 2,2′-bis(3-*sec*-butyl-5-X′-salicylideneamino)biphenyl (B = bp) or -6,6′-dimethylbiphenyl (B = bmp, Y = CH_3), have been shown to undergo the dynamic planar (diamagnetic) $\rightleftharpoons$ tetrahedral (paramagnetic) equilibrium in chloroform solution with mole fraction tetrahedral $N_t^{298°}$ = 0.06–0.12.[4] Unlike the situation with numerous bis-chelate com-

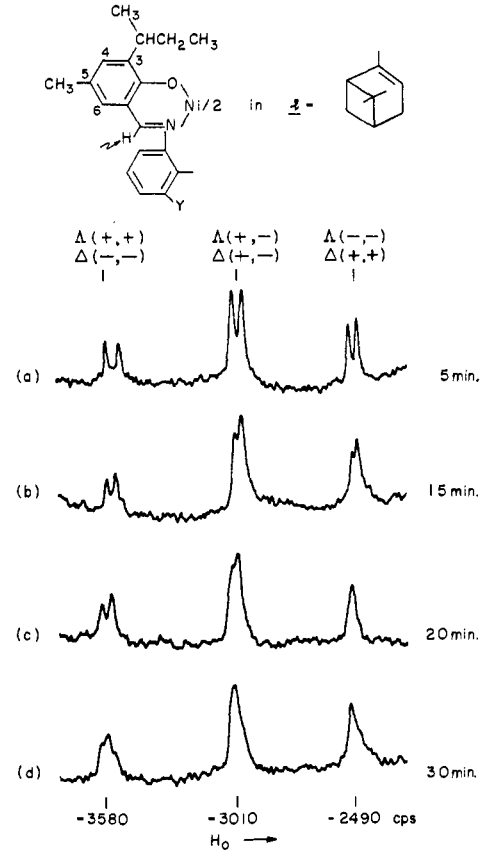


Figure 1. Pmr spectra (60 Mc, ~30°) of the complete isomeric mixture of Ni(3*s*Bu,5Me-sal)$_2$bp (Y = H) in −47.1° α-pinene illustrating the time dependence of the solvent-induced splittings of the azomethine proton signals. Intensities of components of each pair were shown to be equal by alternation of field-sweep direction. Frequencies (cps) are the chemical shifts relative to TMS.

plexes involved in this equilibrium,[1,4–6] the structural interconversion proceeds *without* racemization of the absolute configuration (Δ, Λ[6]) at the metal in the tetrahedral stereoisomers. These configurations are consequently stable on the pmr time scale (and for much longer periods[4]), and the three possible diastereoisomers, Δ(+,+) ≡ Λ(−,−), Δ(−,−) ≡ Λ(+,+), Δ(+,−) ≡ Λ(+,−), are detectable by virtue of three 5-CH_3 and three azomethine proton signals, whose large downfield shifts arise from the contact interaction present in the tetrahedral stereoisomers and whose separations result from unequal ΔF values for the structural change.[4,6] Ni(3*s*Bu,5Me-sal)$_2$bp when dissolved in nearly optically pure *l*-α-pinene[7] ($[\alpha]^{25}D$ −52.2° (neat)) gives a pmr spectrum in the azomethine region very similar to that in Figure 1a. The spectrum differs from that in $CDCl_3$ solution in that each of the three signals is split into a doublet with separations of 80, 50,

(1) Part VI: R. E. Ernst, M. J. O'Connor, and R. H. Holm, *J. Am. Chem. Soc.*, in press.
(2) (a) W. H. Pirkle and S. D. Beare, *ibid.*, **89**, 5485 (1967); (b) W. H. Pirkle and T. G. Burlingame, *Tetrahedron Letters*, 4039 (1967); (c) T. G. Burlingame and W. H. Pirkle, *J. Am. Chem. Soc.*, **88**, 4294 (1966); (d) W. H. Pirkle, *ibid.*, **88**, 1837 (1966).
(3) E. Sackmann, S. Meiboom, and L. C. Snyder, *ibid.*, **90**, 2183 (1968).
(4) M. J. O'Connor, R. E. Ernst, and R. H. Holm, *ibid.*, **90**, 4561 (1968).
(5) R. H. Holm, A. Chakravorty, and G. O. Dudek, *ibid.*, **86**, 397 (1964).
(6) R. E. Ernst, M. J. O'Connor, and R. H. Holm, *ibid.*, **89**, 6104 (1967).
(7) Commercial α-pinene ($[\alpha]^{25}D$ −48.7° (neat), J. T. Baker Chemical Co.) was fractionated on a spinning-band column and further resolved by means of its $AgClO_4$ complex.[8] Pmr spectra of the two samples were identical. The highest reported rotation of an α-pinene enantiomer is stated[9] to be $[\alpha]^{25}D$ +52.4° (neat).[8]
(8) A. E. Comyns and H. J. Lucas, *J. Am. Chem. Soc.*, **79**, 4339 (1957).
(9) D. V. Banthorpe and D. Whittaker, *Chem. Rev.*, **66**, 643 (1966).