[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, BRANDEIS UNIVERSITY, WALTHAM 54, MASS.]

Inversion of Stereospecificity in Hydrolysis by α-Chymotrypsin. The Acetoxyl Substituent[1]

BY SAUL G. COHEN, J. CROSSLEY, EZRA KHEDOURI, ROBERT ZAND AND LUCILLE H. KLEE

RECEIVED SEPTEMBER 24, 1962

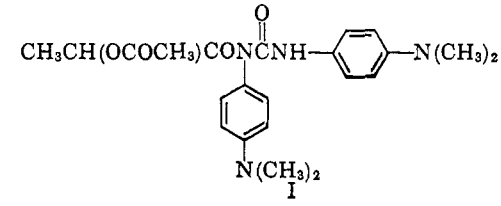
The α-chymotrypsin-catalyzed hydrolyses of some acetoxy-substituted esters have been examined and their stereospecificity compared with that of hydrolyses of corresponding acetamido- and hydroxy-substituted esters. In contrast with ethyl N-acetylalaninate, its nitrogen analog, ethyl α-acetoxypropionate, $CH_3CH(OCOCH_3)CO_2C_2H_5$, shows more rapid hydrolysis of the D-enantiomorph than the L- at concentrations above 0.004 *M*, both when the DL-material and the separate enantiomorphs are examined. The D-compound has less favorable K_m than the L-, 0.18 and 0.0017 *M*, respectively; k_3 is more favorable for the D-compound, 0.020 as compared with 0.00037 sec.$^{-1}$. A mechanism for inversion of antipodal reactivity is described. The corresponding symmetric α-acetoxy compound, diethyl α-acetoxymalonate, $CH_3CO_2CH(CO_2C_2H_5)_2$, is also hydrolyzed with some stereospecificity, while the asymmetric and symmetric β-acetoxy esters, ethyl β-acetoxybutyrate, $CH_3CH(OCOCH_3)CH_2CO_2C_2H_5$, ethyl β-acetoxy-β-phenylpropionate, $C_6H_5CH(OCOCH_3)CH_2CO_2C_2H_5$, and dimethyl β-acetoxyglutarate and diethyl β-acetoxyglutarate, $CH_3CO_2CH(CH_2CO_2R)_2$, are hydrolyzed with no stereospecificity.

Introduction.—Study of the hydrolysis by α-chymotrypsin of esters containing an acetamido group has shown that such compounds are hydrolyzed with stereospecificity when the acetamido group is either in the α- or β-position, and either at a center of asymmetry in compounds of the type Cabde or at a developing center of asymmetry in symmetric compounds of the type Cabdd, in which the symbol d represents an ester group. The symmetric compounds, diethyl α-acetamidomalonate,[2] $CH_3CONHCH(CO_2C_2H_5)_2$, and diethyl β-acetamidoglutarate,[3] $CH_3CONHCH(CH_2CO_2C_2H_5)_2$, were hydrolyzed with stereospecificity leading to optically active half-esters, while diethyl α-acetamidomethylmalonate, $CH_3CONHCH_2CH(CO_2C_2H_5)_2$, in which the acetamido group is not attached directly to the potentially asymmetric carbon atom, showed little or no specificity. Ethyl N-acetylalaninate,[4] $CH_3CONHCH(CH_3)CO_2C_2H_5$, and ethyl β-phenyl-β-acetamidopropionate,[4] $CH_3CONHCH(C_6H_5)CH_2CO_2C_2H_5$, were hydrolyzed stereospecifically, leading to optically active products from DL-starting materials. Ethyl β-acetamidobutyrate, $CH_3CONHCH(CH_3)CH_2CO_2C_2H_5$, and diethyl α-benzyl-α-acetamidomalonate, $CH_3CONHC(CO_2C_2H_5)_2CH_2C_6H_5$, were not hydrolyzed.

Consideration of esters containing a hydroxyl group indicates that this substituent, present in the α- or β-position, may also lead to stereospecificity. The L-enantiomorph of ethyl β-phenyl-α-hydroxypropionate, $C_6H_5CH_2CH(OH)CO_2C_2H_5$, hydrolyzes more rapidly[5] than the D, while ethyl β-phenyl-β-hydroxypropionate, $C_6H_5CH(OH)CH_2CO_2C_2H_5$, and the β-hydroxyglutarates, $HOCH(CH_2CO_2R)_2$, R = CH_3, C_2H_5, were hydrolyzed with stereospecificity.[6] However, ethyl lactate,[6,7] $CH_3CH(OH)CO_2C_2H_5$, and diethyl α-hydroxymalonate, $HOCH(CO_2C_2H_5)_2$, were hydrolyzed without stereospecificity, while ethyl β-hydroxybutyrate, $CH_3CH(OH)CH_2CO_2C_2H_5$, was hydrolyzed exceedingly slowly and non-specifically. The hydroxyl group appears to be more effective in leading to stereospecificity when in the β- than in the α-position, and seems to be assisted in this by an additional large β-substituent, the carbethoxymethylene and phenyl groups of the glutarates and hydroxyhydrocinnamates, respectively, being effective, the methyl and carbethoxyl groups of the lactate, butyrate and malonate esters being ineffective. The β-phenyl group alone may not be effective, the α-chlorohydrocinnamate ester hydrolyzing with no specificity.[8]

Thus, compounds of varied structure, bearing little relationship to the "natural"[9] substrates, may be hydrolyzed stereospecifically by α-chymotrypsin, providing convenient access to many optically active materials. Asymmetric and symmetric compounds of type Cabde and Cabdd, respectively, show similar response of stereospecificity to structural features. A polar interaction, possibly by hydrogen bonding, of the hydroxyl or acetamido group, and a conformational interaction of a second group with an asymmetric grouping in the enzyme may combine to form the diastereomeric transition states or complexes which leave the group which is to be hydrolyzed in contact with or distant from the reactive nucleophilic site. To gain further information about these interactions, it seemed of interest to examine some acetoxy esters, the acetoxyl group bearing relationship to both the acetamido and hydroxyl groups, and lacking the hydrogen atom common to both.

Ethyl DL-α-Acetoxypropionate.—Ethyl DL-α-acetoxypropionate, $CH_3CH(OCOCH_3)CO_2C_2H_5$, was prepared by treatment of ethyl DL-lactate with acetic anhydride.[10] The expected product of hydrolysis, α-acetoxypropionic acid, was prepared by treatment of DL-lactic acid with glacial acetic acid in the presence of sulfuric acid.[10] It was characterized as the substituted ureide I, prepared from 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide,[11] as were the products of subsequent enzymatic hydrolysis of the esters



I

DL, m.p. 153–154°
D and L, m.p. 146–147°
D, $[\alpha]^{22}D$ −50.9°, L, +50.4°, in $CHCl_3$

The DL-ester was subjected to hydrolysis by α-chymotrypsin, 12 mg./ml., in phosphate buffer at *p*H 7.8 in a *p*H-stat. Hydrolysis was fairly rapid initially, slowed down, and was 50% complete after 12 hours, when it apparently stopped. Unhydrolyzed ester was isolated in 85% yield and was characterized by its infrared spectrum which was identical with that of the starting ester. Its optical rotation was determined, α_{obsd} −2.32°, $[\alpha]D^{22}$ −22°, 5.3% in chloroform. Nega-

(1) Requirements for Stereospecificity in Hydrolysis by α-Chymotrypsin, V. For paper IV see ref. 6. For a preliminary report see S. G. Cohen, J. Crossley, E. Khedouri and R. Zand, *J. Am. Chem. Soc.*, **84**, 4163 (1962). We are pleased to acknowledge support of this work by the Division of Research Grants, The National Institutes of Health, RG 4584.

(2) S. G. Cohen and L. H. Klee, *J. Am. Chem. Soc.*, **82**, 6038 (1960).

(3) S. G. Cohen and E. Khedouri, *ibid.*, **83**, 1093 (1961).

(4) S. G. Cohen, Y. Sprinzak and E. Khedouri, *ibid.*, **83**, 4225 (1961).

(5) M. L. Bender and B. W. Turnquest, *ibid.*, **77**, 4271 (1955).

(6) S. G. Cohen and E. Khedouri, *ibid.*, **83**, 4228 (1961).

(7) I. Tinoco, *Arch. Biochem. and Biophys.*, **76**, 148 (1958).

(8) J. E. Snoke and H. Neurath, *Arch. Biochem.*, **21**, 351 (1949).

(9) H. Neurath and G. W. Schwert, *Chem. Rev.*, **46**, 69 (1950).

(10) K. H. Vogel and J. C. Warner, *J. Am. Chem. Soc.*, **75**, 6076 (1953).

(11) F. Zetzche and W. Neiger, *Ber.*, **73B**, 467 (1940).

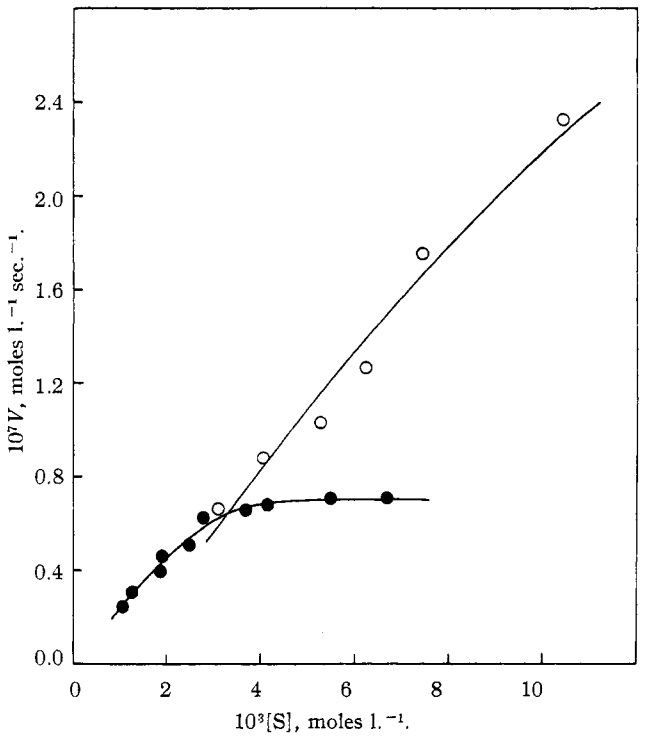


Fig. 1.—Zero-order rates of hydrolysis of L- and D-ethyl α-acetoxypropionate by α-chymotrypsin (5.0 mg./ml.), 0.1 *N* NaCl, *p*H 7.2, 25°: -●-●-●-, L; -O-O-O-, D.

tive rotations were also observed in acetone and ethyl acetate. Since the L-isomer has a negative rotation,[12] excess L-ester was present in the unhydrolyzed material and D-ester in the DL-mixture had been hydrolyzed more rapidly than the L- by the α-chymotrypsin. A synthesized sample of the optically active ethyl acetoxypropionate showed $[\alpha]D^{22}$ −48°, indicating that the ester recovered from the enzymatic hydrolysis contained about 73% L-ester, 27% D, and that the relative rates of hydrolysis of the D- and L-esters were in the ratio of about 2.7 to 1. After the unhydrolyzed ester had been removed, the aqueous hydrolysate was worked up, leading to α-acetoxypropionic acid. This was characterized by its infrared spectrum, and its optical rotation was determined, α_{obsd} +1.77°, $[\alpha]D^{22}$ +23.3°, 3.8% in chloroform. Since the D-acid has a positive rotation,[13] this confirmed the more rapid enzymatic hydrolysis of the D-ester. A synthesized sample of this acid showed $[\alpha]D^{22}$ +49.3° indicating 73% D acid, 27% L in the recovered acid and a relative rate of the hydrolysis of the D- and L-esters of 2.8. The isolated partially active acid was characterized as the substituted ureide I, m.p. 149–150°, $[\alpha]D^{22}$ −16.6°, in chloroform. In another experiment a suspension of a large quantity, 6.75 g., of ethyl DL-α-acetoxypropionate was subjected to the action of 0.200 g. of α-chymotrypsin so that the unhydrolyzed ester could simply be separated, dried, and examined in the polarimeter without the use of solvent. About 15% of the ester was hydrolyzed by the enzyme in 36 hours. The recovered ester was again levorotatory, α_{obsd}^{22} −2.7° 1 dm., and calculation indicated that the relative rates of the hydrolysis of the D- and L-esters were in the ratio of 1.8 to 1.

The Enantiomorphic Ethyl α-Acetoxypropionates.—That the enzymatic hydrolysis of the D-enantiomorph in the racemate is more rapid than that of the L- is of interest, and experiments were carried out on the kinetics of enzymatic hydrolysis of the separate enantiomorphic esters. D(+)- and L(−)-Acetoxypropionic acids were prepared from the corresponding calcium salts of D(−)- and L(+)-lactic acids and characterized by their infrared spectra, boiling points, refractive indices, specific rotations, $[\alpha]D^{22}$ ±49.3°, 7% in chloroform, and by conversion to the optically active ureides. The DL-ureide melted at 153–154°, and a mixture of 2D:1L melted at 151–154°, indicating that the partially active ureide, which had been obtained from the product of enzymatic hydrolysis of the DL-ester, had a satisfactory melting point, 149–151°. The desired ethyl D(+)- and L(−)-α-acetoxypropionates were prepared by treatment of the active acetoxy acids with diazoethane and were characterized by infrared spectra, boiling points, refractive indices and specific rotations, $[\alpha]D^{22}$ +47.7° (D), −47.6° (L), 0.9% in chloroform. A sample of ethyl L(−)-α-acetoxypropionate was also prepared from 40% aqueous L(+)-lactic acid, instead of from calcium lactate, but it seemed to contain some base-consuming impurity, possibly lactide. The kinetic studies were carried out on the optically active esters prepared from the calcium lactates and on the inactive ester prepared from ethyl lactate.

In a preliminary run ethyl L-acetoxypropionate was hydrolyzed in the presence of α-chymotrypsin, and the acid product was isolated and characterized as the ureide I, identical with the product prepared from synthesized L-acetoxypropionic acid, confirming hydrolysis of the desired ester group. As in the case of the DL-ethyl acetoxypropionate, the enzymatic hydrolysis of the separate enantiomorphic esters failed to go to completion, possibly because of inactivation of the enzyme by-product. Preliminary kinetic experiments were carried out at *p*H 7.2, 7.4 and 7.8 in the absence and in the presence of α-chymotrypsin. The nonenzymatic hydrolysis was sensitive to *p*H, being 3.2 times greater at *p*H 7.8 than at 7.2, while the enzymatic rates were comparatively insensitive to *p*H in this range. The subsequent kinetic studies were made at *p*H 7.2, in 0.1 *M* NaCl, 5 mg./ml. of α-chymotrypsin. At each substrate concentration, corrections were applied for non-enzymatic hydrolysis and for consumption of alkali by the enzyme. Details of the procedure are given in the Experimental section. The apparent zero-order enzymatic rates of hydrolysis at the several concentrations of the L-, D-, and DL-substrates are given in Table I and in Fig. 1.

TABLE I

RATES OF HYDROLYSIS OF L-, D- AND DL-ETHYL α-ACETOXYPROPIONATE BY α-CHYMOTRYPSIN (5.0 mg./ml.), 0.1 *M* NaCl, *p*H 7.2, 25°

	[S] × 10^4, mole/l.	*V* × 10^7, mole/l./sec.		[S] × 10^4, mole/l.	*V* × 10^7, mole/l./sec.
L	1.13	0.23	D	1.50	~0
	1.25	.29		3.01	0.57
	1.92	.43		4.07	.89
	1.93	.39		5.30	.90
	2.48	.47		6.20	1.24
	2.84	.60		7.36	1.76
	3.79	.66		10.38	2.33
	4.11	.68	DL	2.25	0.40
	5.54	.72		3.92	.66
	6.69	.71		5.43	.87
				8.23	1.47
				10.00	1.39

At low concentrations the L-enantiomorph was hydrolyzed more rapidly than the D-, but with increasing concentration the rate of hydrolysis of the D-enantiomorph increased much more rapidly, that of the L-enantiomorph leveling off. Our initial preparative

(12) J. Kenyon, H. Phillips and H. G. Turley, *J. Chem. Soc.*, **127**, 399 (1925).

(13) C. M. Bean, J. Kenyon and H. Phillips, *ibid.*, 303 (1936).

hydrolyses were carried out in the presence of excess undissolved ester, at solution concentrations greater than 10^{-1} M, at which concentration the D-enantiomorph is hydrolyzed more rapidly; the kinetic experiments and the preparative experiments with the DL-mixture were consistent. These results differ from those in which a crude liver esterase has been reported to hydrolyze methyl L-mandelate more rapidly than the D when the DL-substrate was studied,[14] and the D-ester more rapidly than the L-when the pure enantiomorphs[15] were used as substrates. The explanation[16] offered for this was that V_{max} was greater for the D-enantiomorph than for the L- while the binding of the L-enantiomorph to the enzyme was the more favorable, the greater affinity of the L-enantiomorph for the enzyme allowing it to be hydrolyzed more rapidly from the DL-material. Presumably the relative values of V_{max} and K_m, the concentration ranges studied and the character of the enzyme–substrate interactions may lead to one effect or the other.

Ethyl L-α-acetoxypropionate appeared to reach its maximum rate of hydrolysis at 5×10^{-3} M, $V_{max} = 0.72 \times 10^{-7}$ mole/l./sec., leading to $k_3 = 0.36 \times 10^{-3}$ sec.$^{-1}$; K_m was determined graphically, 0.0017 mole/l. For the D-enantiomorph a plot of $1/V$ against $1/S$ was linear,[17] and from it the kinetic parameters were determined, $k_3 = 20 \times 10^{-3}$ sec.$^{-1}$, $K_m = 0.18$ mole/l. The kinetic parameters for the DL-material, also determined by the double reciprocal plot,[17] are $k_3 = 3.8 \times 10^{-3}$, $K_m = 0.040$, values intermediate between those of the D- and L-enantiomorphs. A conventional interpretation of this behavior of the D- and L-compounds is that the L-enantiomorph is more firmly bound (smaller K_m) while the D-enantiomorph has a higher specific rate of hydrolysis (k_3). We propose that this results from the nature of the preferred mode of binding of this substrate to α-chymotrypsin, in which the more common mode of association of the L-enantiomorph does not place the carbethoxyl group at the active nucleophilic site.

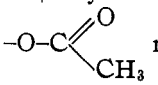
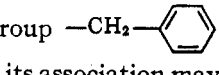
If this substrate complexed preferentially with the enzyme in such a way that the acetoxyl substituent and α-hydrogen had the same association and orientation as the acetamido group and α-hydrogen in ethyl acetylalaninate, or ethyl N-acetylphenylalaninate,[9] the L-enantiomorph would place the carbethoxyl group at the nucleophilic or essential catalytic site, and we believe that a higher rate of hydrolysis of this enantiomorph would result, since this enantiomorph is the more firmly bound. This would lead to normal or "natural" stereospecificity; but this is not found. However, it may be that the acetoxyl group, lacking the H atom and thus the polar association properties of the acetamido group, may associate either at the polar acylamido contact site or at the non-polar β-aryl contact site of α-chymotrypsin. The group  may in this situation act more like the group  than like the acylamido, and much of its association may be at the non-polar β-aryl contact site, and it may do this more effectively than the simple end-methyl group. Also the α-hydrogen may well assume one required conformational relation in association of substrates with this enzyme, fitting into a small space, since substrates with a quaternary α-carbon are generally hydrolyzed very slowly if at all by α-chymotrypsin.[18] On this basis, associations of substrates with α-chymotrypsin are described in the following scheme, in which the enzyme is indicated schematically as providing association sites in an expanded tetrahedral arrangement (Chart I).

CHART I

a = non-polar (β-aryl) contact site (enzyme)
am = acylamido contact site (enzyme)
n = nucleophilic or catalytic site (enzyme)
Am = acetamido group (substrate)
Ao = acetoxyl group (substrate)

Substrate Enzyme

II, R = H, ethyl L-acetylalaninate
R = C_6H_5, ethyl L-N-acetylphenylalaninate

III, ethyl L-α-acetoxypropionate

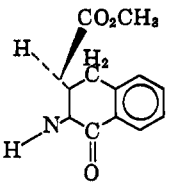
IV, ethyl L-α-acetoxypropionate

V, ethyl D-α-acetoxypropionate

In II are indicated normal associations of ethyl L-acetylalaninate or ethyl L-N-acetylphenylalaninate which are leading to effective attack of the reactive enzyme group n upon the ester. In III is indicated the corresponding association of ethyl L-acetoxypropionate which would lead to hydrolysis of this enantiomorph. However, we suggest that the dominant form of association of this enantiomorph may be indicated by IV with the acetoxyl group associated with the non-polar contact site a, and the ester group thus associated with the polar acylamido contact site am; this may lead to favorable K_m, which may be the resultant of both modes of association III and IV, but in conformation IV reaction does not result. In a sense, association mode IV corresponds to the tight complexing of D-enantiomorphs of natural substrates which do not lead to subsequent reaction but to inhibition.[19] To the extent the K_m reflects this non-reacting assocation, it may be a true equilibrium constant. In V is indicated the analogous association of the D-enantiomorph, with the acetoxyl group associated with the non-polar contact site a and the ester group now accessible for reaction with n. This may have a less favorable K_m than IV due to the low association of the methyl group with the acylamido site am, but with increasing concentration the rate of reaction may rise, as found.

A detailed and general analysis of reactivity and stereospecificity, involving productive and non-productive binding, has been made by Hein and Niemann[20a,b] and it has been applied to the hydrolysis of 1-keto-3-carbomethoxytetrahydroisoquinoline (VI) by α-chymotrypsin.

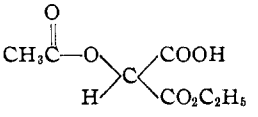
(14) P. Rona and E. Chain, *Biochem. Z.*, **258**, 480 (1933).
(15) P. Rona and R. Ammon, *ibid.*, **181**, 49 (1927).
(16) H. H. Weber and R. Ammon, *ibid.*, **204**, 197 (1929).
(17) H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, **56**, 658 (1934).
(18) H. R. Almond, Jr., D. T. Manning and C. Niemann, *Biochemistry*, **1**, 243 (1962).
(19) H. T. Huang and C. Niemann, *J. Am. Chem. Soc.*, **73**, 3223 (1951).
(20) (a) G. E. Hein and N. Niemann, *Proc. Natl. Acad. Sci.*, **47**, 1341 (1961); (b) *J. Am. Chem. Soc.*, **84**, 4495 (1962).



VI

Hydrolysis of VI was the first example[21a,b] of inversion of antipodal specificity of α-chymotrypsin, the D-enantiomorph hydrolyzing very rapidly and much faster than the L. The rigid bicyclic nucleus is considered to be important in leading to this behavior.[20] Other interpretations of the steric factors involved in the hydrolysis of VI have been offered,[22a,b] in each case depending upon the cyclic nature and the constrained conformations of this substrate. Ethyl α-acetoxypropionate appears to be the first non-cyclic sterically unconstrained ester which shows inversion of the usual antipodal specificity in hydrolysis by α-chymotrypsin. Replacement of the NH group in the alanine derivative by an oxygen atom in our substrate leads to inversion of stereospecificity, probably because of a different preferred association between substrate and enzyme, and the difference does not appear to be related to size.

Diethyl α-Acetoxymalonate.—Since we wish to continue comparison of stereospecificity in α-chymotrypsin-catalyzed reactions of related asymmetric and symmetric substrates of the types Cabde and Cabdd, respectively, the symmetric compound diethyl α-acetoxymalonate, $CH_3COOCH(CO_2C_2H_5)_2$, was studied, in which one carboethoxy group replaces the methyl group of ethyl α-acetoxypropionate. This compound was prepared by acetylation of diethyl α-hydroxymalonate, which had been prepared and studied previously.[6] It was hydrolyzed by α-chymotrypsin considerably more rapidly than was ethyl α-acetoxypropionate, perhaps consistent with its greater chemical reactivity. The hydrolysate was optically active, α_{obsd} +0.55°, the rotation decreasing with time, the product, 2-acetoxy-2-carboethoxyacetic acid



racemizing much as the corresponding acetamido compound had.[2] The product in this case was an oil, obtained in high yield, but characterized only by its infrared spectrum. Repeated attempts to prepare a solid derivative by treatment with 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide failed. The observed positive sign of rotation in the hydrolysate is the same as that found in the hydrolysis of diethyl α-acetamidomalonate,[2] but one would hesitate to comment on the absolute configuration. The α-acetoxy substituent in both the symmetric and asymmetric esters led to some stereospecificity, while the α-hydroxy substituent in the corresponding lactate and α-hydroxymalonate esters had led to no stereospecificity.

β-Acetoxy Esters.—As in the cases of the acetamido and hydroxy esters, it seemed desirable to compare the effectiveness of the acetoxyl group in the α- and β-positions in leading to stereospecificity.

(21) (a) G. E. Hein, R. B. McGriff and C. Niemann, *J. Am. Chem. Soc.*, **82**, 1830 (1960); (b) G. E. Hein and C. Niemann, *ibid.*, **84**, 4487 (1962).

(22) (a) E. S. Awad, H. Neurath and B. S. Hartley, *J. Biol. Chem.*, **235**, PC 35 (1960); (b) I. B. Wilson and B. F. Erlanger, *J. Am. Chem. Soc.*, **82**, 6422 (1960).

Ethyl *dl*-β-acetoxybutyrate was prepared by acetylation of the β-hydroxy ester. It was hydrolyzed very slowly by α-chymotrypsin and with no stereospecificity. Both the recovered unhydrolyzed ester and the isolated hydrolysis product, β-acetoxybutyric acid, and the ureide of the latter from 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide were optically inactive.

Dimethyl and diethyl β-acetyoxyglutarates were prepared by acetylation of the β-hydroxyglutarates. They were hydrolyzed readily but non-stereospecifically by α-chymotrypsin, leading to the optically inactive β-acetoxy half-esters in high yield. These were obtained as oils, identified by infrared spectra and characterized as their inactive solid ureide derivatives by treatment with 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide. These hydrolyses stand in contrast with the hydrolyses of the β-hydroxy-[6] and β-acetamidoglutarates[3] by α-chymotrypsin which proceed with complete stereospecificity. Furthermore, consideration of the time required for complete hydrolysis of one ester group in each of these glutarates indicates that the present non-stereospecific hydrolyses of the β-acetoxy esters proceed considerably more rapidly than the stereospecific hydrolyses of the β-hydroxy-[6] and β-acetamidoglutarates.[3]

Finally, ethyl *dl*-β-acetoxy-β-phenylpropionate was prepared by acetylation of the corresponding β-hydroxy ester, which had been prepared previously and found to be hydrolyzed stereospecifically[6] by α-chymotrypsin. This acetoxy ester is now found to be hydrolyzed slowly by α-chymotrypsin, with no stereospecificity, despite the presence of the β-aryl substituent, characteristic of natural substrates.[9] Optical activity was found in neither the recovered unhydrolyzed ester, nor in the hydrolysis product, β-acetoxy-β-phenylpropionic acid, nor in the ureide prepared from this by treatment with 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide.

The acetoxyl group shows a similar effect, or lack of effect, in the hydrolysis of both symmetric and asymmetric esters by α-chymotrypsin. When in the α-position it leads to some stereospecificity in the hydrolysis of the malonate and of the acetoxypropionate; in the latter case this may be accompanied by inversion of antipodal specificity. In the β-position it leads to no stereospecificity, neither in the butyrate, nor in the β-phenylpropionate, nor in the glutarate. The hydroxyl substituent also behaved similarly in symmetric and asymmetric esters, but shows high effectiveness in leading to stereospecificity when in the β-position, and less effectiveness when in the α-position. The acetamido substituent shows high effectiveness in leading to stereospecificity, whether in the α- or in the β-position and whether in symmetric or in asymmetric compounds, providing it is attached directly to a center or to a developing center of asymmetry.

Experimental

Melting points are uncorrected. Elementary analyses are by Dr. S. M. Nagy, Massachusetts Institute of Technology, unless otherwise indicated.

1,3-Bis-(*p*-dimethylaminophenyl)-carbodiimide was prepared as described previously,[6] by treatment of N,N-dimethyl-*p*-phenylenediamine with carbon disulfide, leading to N,N′-bis-(*p*-dimethylaminophenyl)-thiourea, followed by treatment of this with lead oxide in the presence of flowers of sulfur; m.p. 87–90°, reported[11] 88–90°.

α-Chymotrypsin was obtained from Worthington Biochemical Corporation, salt-free, recrystallized. Samples were dried to constant weight at 120° for assay of protein content. The molecular weight was assumed to be 25,000 for calculation of kinetic parameters.

Optical rotations were determined in a Zeiss-Winkel polarimeter and read to ±0.02°.

Infrared spectra were determined on a Perkin-Elmer model 21 instrument.

Ethyl DL-α-Acetoxypropionate.—A solution of 50 g. (0.42 mole) of DL-ethyl lactate (Matheson, Coleman and Bell) and 100 g. (1.0 mole) of acetic anhydride (Baker A.R.) was boiled under reflux for 2.5 hours and acetic acid and excess acetic anhydride were boiled off at atmospheric pressure. The residue was washed with three 50-ml. portions of distilled water, dissolved in 50 ml. of ether and washed three more times, dried over sodium sulfate and distilled, leading to ethyl DL-α-acetoxypropionate, 22.4 g. (0.14 mole), 33% yield, b.p. 81–82° (25 mm.), $n^{22}D$ 1.4050; reported[10] b.p. 175–177° (745 mm.), $n^{28}D$ 1.4051.

DL-α-Acetoxypropionic Acid.—A solution of 121 g. (1.2 moles) of 85–90% DL-lactic acid (Merck and Co.), 540 g. (9.0 moles) of glacial acetic acid, 1 ml. of concentrated sulfuric acid and 200 ml. of benzene was boiled under reflux for 24 hours, water being removed with a Dean–Stark trap. The residue was concentrated and distilled, leading to DL-α-acetoxypropionic acid, 102 g. (0.77 mole), 64% yield, b.p. 108–109° (1.5 mm.), $n^{25}D$ 1.4220, m.p. 43.5–45.5°; reported b.p. 101° (1.5 mm.), $n^{28}D$ 1.4222,[10] m.p. 57–60°,[23a] 38–40°.[23b]

A solution of 0.50 g. (3.8 mmoles) of DL-α-acetoxypropionic acid in 10 ml. of ether was treated with a solution of 1.06 g. (3.8 mmoles) of 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide in 20 ml. of ether under reflux for 3 hours. The mixture was cooled in a freezer and filtered, leading to **1-(DL-2-acetoxypropanoyl)-1,3-bis-(*p*-dimethylaminophenyl)-urea**, 1.10 g. (2.7 mmoles), 70% yield, m.p. 153–154°, from chloroform–ether.

Anal. Calcd. for $C_{22}H_{28}O_4N_4$: C, 64.06; H, 6.84; N, 13.58. Found: C, 63.04; H, 6.60; N, 13.43.

L(−)-α-Acetoxypropionic Acid.—A solution of 25 g. of 40% aqueous L(+)-lactic acid (Mann Research Laboratories, 0.11 mole), 70 g. (1.16 moles) of glacial acetic acid, 0.5 ml. of concentrated sulfuric acid and 100 ml. of benzene was boiled under reflux for 18 hours, water being removed. Sodium acetate, 2 g., was added and the solution was concentrated and distilled, leading to L(−)-α-acetoxypropionic acid, 7.3 g. (0.055 mole), 50% yield, b.p. 115–117° (2 mm.), $n^{25}D$ 1.4220, $[\alpha]^{22}D$ −49.3°, 7.3% in $CHCl_3$; reported[13] b.p. 136–137° (16 mm.), $[\alpha]^{18}D$ −47.8° (no solvent).

Anal. Calcd. for $C_5H_8O_4$: C, 45.45; H, 6.10. Found: C, 45.82; H, 5.80.

A sample of this compound was converted to **1-(L(+)-2-acetoxypropanoyl)-1,3-bis-(*p*-dimethylaminophenyl)-urea** by treatment with the carbodiimide as described for the DL-compound, 86% yield, soften 144°, m.p. 146–147°, from chloroform–ether, $[\alpha]^{22}D$ + 50.4°, 5.12% in chloroform.

Anal. Found: C, 63.98; H, 6.81; N, 13.47.

A second portion of **L(−)-α-acetoxypropionic acid** was prepared, as described below for the D-compound, from 15 g. (0.091 mole) of the calcium salt, tetrahydrate, of L(+)-lactic acid (Mann Research Laboratories). This led to 4.1 g. (0.031 mole) of L(−)-α-acetoxypropionic acid, 34% yield, b.p. 110–114° (1 mm.). This was converted directly to the ethyl ester (below).

D(+)-α-Acetoxypropionic Acid.—A solution of 5 g. (0.030 mole) of the calcium salt, tetrahydrate, of D(−)-lactic acid (Mann Research Laboratories) in 15 ml. of water was treated with 100 ml. of glacial acetic acid and 2 g. (0.02 mole) of concentrated sulfuric acid. Calcium sulfate was filtered and washed with acetic acid and the filtrates were combined. A second 5-g. portion of calcium lactate was treated similarly and the acetic acid solutions were combined, treated with 200 ml. of benzene and 2 ml. of concentrated sulfuric acid and boiled under reflux for 24 hours, water being removed with a Barrett trap. Sodium acetate, 3 g., was added, the solution was concentrated, filtered and distilled, leading to D(+)-α-acetoxypropionic acid, 5.9 g. (0.045 mole), 75% yield, b.p. 112–113° (1 mm.), $n^{25}D$ 1.4220, $[\alpha]^{22}D$ +49.3°, 6.92% in chloroform.

A sample of this was converted to **1-(D(−)-2-acetoxypropanoyl)-1,3-bis-(*p*-dimethylaminophenyl)-urea** by treatment with the carbodiimide as described for the DL-compound; 80% yield, soften 144°, m.p. 146–147°, from chloroform–ether, $[\alpha]^{22}D$ −50.9°, 4.01% in chloroform.

Anal. Found: C, 63.84; H, 6.67; N, 13.76.

Diazoethane.—Potassium hydroxide, 30 g., was dissolved in 120 ml. of ethanol, diluted with 100 ml. of ether and warmed until the ether began to distil. N-Nitrosoethylurethane,[24] 30 g. (0.23 mole) in 100 ml. of ether, was added rapidly and diazoethane was distilled over with the ether and condensed in salt–ice. Distillation was continued until the distillate was colorless. Ether was added to the reaction flask during the distillation to maintain its volume there at a minimum of 100 ml.

Ethyl D(+)-α-Acetoxypropionate.—D(+)-α-Acetoxypropionic acid (4.5 g., 0.034 mole) was added to diazoethane in ether (prepared above from 30 g. of nitrosoethylurethane) and allowed to stand for 2 hours. The solution was dried over magnesium sulfate, concentrated and distilled, leading to ethyl D(+)-α-acetoxypropionate, 1.63 g. (0.010 mole), 30% yield, b.p. 63° (12 mm.), $n^{25}D$ 1.4079, $[\alpha]^{22}D$ +44.6° (no solvent); +47.7°, 0.91% in chloroform; +62.9°, 3.97% in ethanol; reported[12] +49.8°.

Anal. Calcd. for $C_7H_{12}O_4$: C, 52.50; H, 7.51. Found: C, 52.71; H, 7.58 (by A. Bernhardt, Mulheim).

Ethyl L(−)-α-Acetoxypropionate.—L(−)-α-Acetoxypropionic acid (4.1 g., 0.031 mole), prepared from calcium lactate, was added to diazoethane and worked up as described above for the D-compound, leading to ethyl L(−)-α-acetoxypropionate, 1.80 g. (0.011 mole), 36% yield, b.p. 45–48° (8 mm.), $n^{23}D$ 1.4074, $[\alpha]^{22}D$ −44.7° (no solvent); −47.6°, 0.90% in chloroform; −62.9°, 4.45% in ethanol; reported[12] $n^{19}D$ 1.4095, $[\alpha]^{18}D$ −47.6°.

Anal. Found: C, 51.8; H, 7.6 (by Dr. C. Fitz).

Hydrolysis of Ethyl DL-α-Acetoxypropionate by α-Chymotrypsin.—(i) The ester 1.101 g. (6.88 mmoles), 2.5 ml. of 0.1 *M* Na_2HPO_4, 0.300 g. of the enzyme and 22 ml. of distilled water were adjusted to *p*H 7.8 and the hydrolysis was followed in a *p*H-stat, *p*H 7.8, 27°, with magnetic stirring, *N* NaOH being added from an automatic buret. The ester was not completely in solution initially. Alkali was consumed fairly rapidly initially, 0.82 mmole in the first hour, 0.57 in the second, 2.5 after 5 hours, 3.4 mmoles after 12 hours, then slowing down markedly, despite the addition of 0.200 g. of fresh enzyme. The solution was saturated with sodium chloride and extracted with five 30-ml. portions of ether. The extract was dried over sodium sulfate and concentrated, leaving a colorless liquid, 0.475 g. (3.0 mmoles), 85% yield. The infrared spectrum was identical with that of the starting ester with peaks at 3.35(w), 5.70(s), 6.89(w), 7.26(m), 7.64(w), 8.05(m), 8.30(m), 8.81(w), 9.05(m), 9.50(w), 9.80(w) μ. The optical rotation was determined, α_{obsd} −2.32°, 2 dm., 5.3% in chloroform, $[\alpha]^{22}D$ −22°. The aqueous hydrolysate, which had been extracted, was brought to *p*H 2 with *N* HCl, concentrated to a small volume *in vacuo* and extracted with five 100-ml. portions of ether. The extract was dried and concentrated, leaving a colorless sirup, 0.385 g. (2.9 mmoles), 85% yield calculated for α-acetoxypropionic acid, α_{obsd} +1.77°, 2 dm., 3.8% in chloroform, $[\alpha]^{22}D$ +23.3°, indicating 47% D-acid, 53% DL in the recovered acid. A portion of this (0.100 g., 0.76 mmole) in 10 ml. of ether was treated with 0.210 g. (0.77 mmole) of 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide in 15 ml. of ether under reflux for 3 hours and cooled at 0°, leading to 1-(2-acetoxypropanoyl)-1,3-bis-(*p*-dimethylaminophenyl)-urea, 0.22 g. (0.54 mmole), 71% yield, m.p. 149–151°, from acetone–ether, α_{obsd} −0.38°, 1 dm., 2.29% in chloroform, $[\alpha]^{22}D$ −16.6°, indicating excess of D(−)-ureide.

Anal. Found: C, 63.94; H, 6.95; N, 13.62.

(ii) The enzymatic hydrolysis of ethyl DL-α-acetoxypropionate was repeated with quantities and conditions identical with those of experiment (i) above, leading to 0.45 g. (2.8 mmoles), 81% recovery, of the unhydrolyzed ester. This was dissolved in 0.45 ml. of chloroform and its rotation determined in a 1-dm. tube, α^{22}_{obsd} −18.45°, $[\alpha]^{22}D$ −17.7°, indicating 35% excess of L(−)-ester.

(iii) A suspension of 6.75 g. (42.2 mmoles) of ethyl DL-α-acetoxypropionate in 20 ml. of distilled water containing 0.200 g. of α-chymotrypsin and 2.5 ml. of 0.1 *M* Na_2HPO_4 was allowed to react in the *p*H-stat, *p*H 7.8, for 36 hours, 9.85 ml. of *N* NaOH being consumed. Blank runs indicated consumption of 0.42 ml. by the enzyme and of 2.88 ml. by the non-enzymatic hydrolysis during this period; net consumption of alkali due to enzymatic hydrolysis, 6.55 ml. (6.55 mmoles). The suspended unreacted ester was separated and dried, 3.8 g. (24 mmoles), α^{22}_{obsd} −2.7°, 1 dm.

Effect of *p*H on Rate of Hydrolysis of Ethyl α-Acetoxypropionate. (i) **Non-enzymatic Hydrolysis.**—A saturated solution (0.145 *M*) of the ester in 20 ml. of 0.0056 *M* phosphate was allowed to consume alkali in the *p*H-stat. The rates of consumption of 0.2 *N* NaOH were: *p*H 7.2, 0.0006 ml., 0.00012 mmole, 0.004%/min.; *p*H 7.4, 0.00087 ml., 0.00017 mmole, 0.0058%/min.; *p*H 7.8, 0.0019 ml., 0.00037 mmole, 0.013%/min.

(ii) **Enzymatic Hydrolysis.**—A solution of 0.207 g. (1.29 mmoles, 0.06 *M*) of ethyl DL-α-acetoxypropionate, 2 ml. of 0.1 *M* Na_2HPO_4 and 0.100 g. of α-chymotrypsin in 20 ml. of water was allowed to consume alkali. Initial rates of consumption of 0.2 *N* NaOH were: *p*H 7.2 and 7.4, 0.0140 ml., 0.0028 mmole, 0.22%/min.; *p*H 7.8, 0.0146 ml., 0.0029 mmole, 0.22%/min.

Hydrolysis of Ethyl L(−)-α-Acetoxypropionate by α-Chymotrypsin.—A solution of 0.483 g. (3.00 mmoles) of the ester and 0.205 g. of the enzyme in 20 ml. of 0.1 *M* NaCl was allowed to hydrolyze in the *p*H-stat at *p*H 7.2, 0.1 *N* NaOH being added. The reaction was about 50% complete in 24 hours. The unreacted ester was extracted with ether; the aqueous solution was brought to *p*H 6 and lyophilized. The residue was extracted with 30-ml. portions of ether and the extracts were dried and concentrated. The residue, L(−)-α-acetoxypropionic acid, was treated

(23) (a) R. Anschütz and W. Bertram, *Ber.*, **37**, 3967 (1904); (b) M. V. Auger, *Compt. rend.*, **140**, 938 (1905).

(24) W. H. Hartman and R. Phillips, "Organic Syntheses," Coll. Vol. II, A. H. Blatt, editor, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 464.

with 0.28 g. of 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide in 20 ml. of ether under reflux for 3 hours, leading to a precipitate of the ureide, 0.237 g., m.p. 143–146° crude, m.p. 146–147° from chloroform–ether; mixture m.p. with an authentic sample, 146–147°; $[\alpha]^{22}D$ +48.6°, 2.22% in chloroform.

Kinetics of Enzymatic Hydrolysis of Ethyl L-, D-, and DL-α-Acetoxypropionate.—The rates of hydrolysis were followed in a *p*H-stat, at 25.0 ± 0.1°, the *p*H being maintained at 7.2 with a Radiometer Titrator, model TTT 1B, 0.1 *N* NaOH being delivered from an Aminco automatic buret. The reactions were run in a 30-ml. beaker equipped with water-jacket, Teflon-coated magnetic stirrer and a plastic cover through which passed the electrodes, the buret tip, a gas inlet tube and a sample holder. Sodium chloride solution, 0.1 *M*, 15 ml., was run into the beaker; the ester was weighed into a small glass tube and suspended from the holder; nitrogen gas was bubbled through distilled water and passed over the solution; stirring was started, the solution was brought to *p*H 7.2, the ester was dropped in and the rate of water hydrolysis was followed for 40 minutes. The enzyme solution, 0.100 g. of α-chymotrypsin in 5 ml. of 0.1 *M* NaCl, was added, the *p*H was brought rapidly back to 7.2 and the total rate of hydrolysis was followed to about 15% hydrolysis. Plots of added alkali *vs.* time, corrected for non-enzymatic hydrolysis and for consumption of alkali by the enzyme, were linear over the periods examined.

Diethyl α-Acetoxymalonate.—Diethyl α-hydroxymalonate[6] (20 g., 0.113 mole) was treated with 40 g. of acetic anhydride at 100° for 2 hours. The product was isolated by distillation, b.p. 132–134° (15 ml.), reported[25] 114–116° (5 mm.), 17 g. (0.078 mole), 69% yield.

A suspension of 1.472 g. (6.75 mmoles) of this ester in a solution of 2.5 ml. of 0.1 *M* Na_2HPO_4 and 0.200 g. of α-chymotrypsin in 20 ml. of water was brought to *p*H 7.8 and followed in the *p*H-stat at 28°. After 4.5 hours 6.68 ml. of *N* NaOH was consumed, 99% hydrolysis of one ester group, of which a blank indicated 14% was due to hydroxide, the balance, 85%, being due to the enzyme. The solution was examined in a polarimeter and compared with a chymotrypsin blank, showing a net contribution due to the product of +0.55°, which fell after 0.5 hour to +0.41° and after 16 hours to +0.15°. The solution was brought to *p*H 2 with *N* HCl, taken to dryness *in vacuo* at room temperature and extracted with ether. The ether was removed, leading to an oil, 1.25 g. (6.6 mmoles), 99% yield of ethyl hydrogen-α-acetoxymalonate; it showed infrared absorption bands in chloroform at: 2.90(w), 3.10(w), 3.45(m), 5.75(s), 6.96(m), 7.32(m), 8.50(m), 9.15(m), 9.70(m), 11.75(w) μ.

DL-Ethyl β-acetoxybutyrate was prepared by treatment of 25 g. (0.21 mole) of *dl*-ethyl-β-hydroxybutyrate (Eastman Kodak Co., b.p. 179°) with 50 g. (0.5 mole) of acetic anhydride at 100° for 2 hours. The solution was distilled, leading to the product, 29 g. (0.17 mole), 80% yield, b.p. 92–94° (8 mm.), reported[26] 72° (0.4 mm.). The infrared spectrum was obtained in chloroform: 3.34(w), 5.75(s), 6.92(w), 7.25(m), 7.65(m), 8.05(s), 8.77(w), 9.05(w), 9.40(m), 9.70(m) μ.

A suspension of the ester (1.011 g., 5.8 moles) in a solution of 2 ml. of 0.1 *M* Na_2HPO_4 and 0.300 g. of α-chymotrypsin in 20 ml. of water was brought to *p*H 7.8 and followed in the *p*H-stat at 30°. After 13 hours, 1.83 ml. of *N* NaOH had been consumed, 31.5% hydrolysis, some ester still remaining undissolved. The mixture was extracted with ether and the extracts were dried and concentrated, leading to recovered *dl*-ethyl β-acetoxybutyrate (0.625 g., 3.6 mmoles), 90% recovery, α_{obsd} 0.00°, 6.8% in chloroform. Its infrared spectrum in chloroform was identical with that of the starting material. The solution which had been extracted was brought to *p*H 2 and taken to dryness *in vacuo* and the residue was extracted with acetone. Evaporation of the acetone led to *dl*-β-acetoxybutyric acid, 0.24 g. (1.64 mmoles), 96% yield, α_{obsd} 0.00°, 2.2% in chloroform. A solution of this acid, 0.24 g. (1.64 mmoles) in 10 ml. of ether, was added to a solution of 0.46 g. (1.64 mmoles) of 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide in 15 ml. of ether and boiled under reflux for 3 hours. The mixture was filtered, leading to **1-(DL-3-acetoxybutanoyl)-1,3-bis-(*p*-dimethylaminophenyl)-urea**, 0.57 g. (1.34 mmoles), 82% yield, m.p. 159–160°, from chloroform–ether, α_{obsd} 0.00°, 1.8% in chloroform.

Anal. Calcd. for $C_{22}H_{28}O_4N_4$: C, 64.77; H, 7.09; N, 13.14. Found: C, 64.41; H, 7.15; N, 13.59.

DL-Methyl Hydrogen β-Acetoxyglutarate.[27]—Dry crude dipotassium β-hydroxyglutarate, prepared from 10 g. (0.057 mole) of dimethyl β-hydroxyglutarate,[6] was treated at 0° with 17 g. (0.20 mole) of acetyl chloride. The temperature was allowed to rise to 35° and was kept there for 1 hour. Excess acetyl chloride was removed *in vacuo*, and the residue was extracted with hot chloroform. The extract was filtered and concentrated *in vacuo* to 15 ml., diluted with 5 ml. of ether and cooled, leading to 5.2 g. (0.033 mole), 54% yield, of β-acetoxyglutaric anhydride, m.p. 84–86°, reported[27] 86°. This anhydride (3.1 g., 0.018 mole) and methanol (1.8 g., 0.059 mole) were heated under reflux for 0.5 hour, concentrated and treated with 7 ml. of water. Concentrated ammonia–water (1:2) was added until the smell of ammonia became perceptible. The solution was extracted with ethyl acetate to remove any diester, acidified and again extracted with ethyl acetate. The latter extract was dried and concentrated *in vacuo*, leading to methyl hydrogen α-acetoxyglutarate, a light yellow oil, 2.2 g. (0.014 mole), 78% yield. The infrared spectrum was determined in chloroform: 2.90(w), 3.44(m), 5.80(s), 6.97(m), 7.30(m), 8.40(m), 9.72(m), 10.55(w) μ. A portion of this half-acid (0.31 g., 1.52 mmoles) was treated, in 30 ml. of ether, with 0.42 g. (1.52 mmoles) of 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide. The solution was boiled under reflux for 3 hours and cooled in ice, leading to **1-(DL-3-acetoxy-4-methoxycarbonylbutanoyl)-1,3-bis-(*p*-dimethylaminophenyl)-urea**, 0.55 g. (1.15 mmoles), 76% yield. This was treated with Norit A in chloroform, taken to dryness and recrystallized from 3:1 ether–petroleum ether; colorless plates, m.p. 131–132°.

Anal. Calcd. for $C_{25}H_{32}O_6N_4$: N, 11.56. Found: N, 11.57.

Dimethyl β-Acetoxyglutarate.—Dimethyl β-hydroxyglutarate[6,28] (15 g., 0.085 mole) was heated on the steam-bath for 2 hours with 15 g. (0.18 mole) of sodium acetate and 30 g. (0.30 mole) of acetic anhydride and concentrated *in vacuo*. The residue was extracted with ether, the extract was dried and concentrated and the product was distilled; 16 g. (0.074 mole), 91% yield, b.p. 145–147° (8 mm.), reported[28] 134–135° (8 mm.).

A suspension of 1.26 g. (5.8 mmoles) of dimethyl β-acetoxyglutarate, 2.0 ml. of 0.1 *M* Na_2HPO_4 and 0.200 g. of α-chymotrypsin in 25 ml. of water was brought to *p*H 7.8 and followed in the *p*H-stat at 27°. The material went into solution as the hydrolysis proceeded, 3.0 ml. of *N* NaOH being consumed in 1 hour, 52% hydrolysis, and 5.8 ml. in 5 hours, 100% hydrolysis of one ester group. Hydroxide ion-catalyzed hydrolysis at *p*H 7.8 was negligible. The optical rotation of the solution was compared with α-chymotrypsin blank and showed no contribution due to the reaction. The solution was brought to *p*H 2 and taken to dryness *in vacuo* at room temperature. The residue was extracted with ether, the extract was dried and concentrated, leaving as an oil DL-methyl hydrogen β-acetoxyglutarate, 0.95 g. (4.65 mmoles), 80% yield; its infrared spectrum in chloroform was identical with that of the synthetic sample, α_{obsd} 0.00°, 10% in chloroform. A solution of 0.16 g. (0.75 mmole) of this in 5 ml. of ether was treated with 0.21 g. (0.77 mmole) of 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide as described above, leading to the ureide, 0.26 g. (0.54 mmole), 72% yield, m.p. and mixture m.p. with the authentic sample 131–132°, α_{obsd} 0.00°, 1.72% in chloroform.

Diethyl β-acetoxyglutarate was prepared by treatment of 30 g. (0.15 mole) of diethyl β-hydroxyglutarate with 60 g. of acetic anhydride under reflux for 1 hour and isolated by distillation; 31 g. (0.13 mole), 86% yield, b.p. 153–154° (11 mm.), reported[28] b.p. 138° (4 mm.). Two grams (8.2 mmoles) of this was hydrolyzed by 0.100 g. of α-chymotrypsin, as described above, complete hydrolysis of one ester group requiring 10 hours. There was no optical activity due to the product.

Ethyl hydrogen β-acetoxyglutarate was isolated as described above, 1.65 g. (7.6 mmoles), 93% yield, and found to be optically inactive. A portion (0.30 g., 1.4 mmoles) was treated with 0.39 g. (1.4 mmoles) of 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide leading to the ureide, 0.62 g., 89% yield.

Anal. Calcd. for $C_{26}H_{34}O_6N_4$: C, 62.63; H, 6.87; N, 11.24. Found: C, 62.55; H, 7.07; N, 11.30.

DL-Ethyl β-acetoxy-β-phenylpropionate was prepared by treatment of 15 g. (0.078 mole) of DL-ethyl β-hydroxy-β-phenylpropionate[6,29] with 20 g. of acetic anhydride at 100° for 2 hours. The product was distilled, 16 g. (0.068 mole), 88% yield, b.p. 184–186° (3 mm.). The infrared spectrum was obtained in chloroform: 3.40(m), 5.578(s), 6.70(w), 6.90(w), 7.30(m), 8.35(m), 9.30(m), 9.80(m), 10.60(w) μ.

Anal. Calcd. for $C_{13}H_{16}O_4$: C, 66.08; H, 6.83. Found: C, 66.04; H, 6.81.

A suspension of 1.25 g. (5.3 mmoles) of the ester in a solution of 2.5 ml. of 0.1 *M* Na_2HPO_4 and 0.300 g. of α-chymotrypsin in 20 ml. of water was brought to *p*H 7.8 and followed with the *p*H-stat at 28°. After 24 hours, 1.22 ml. of *N* NaOH was consumed, 23% hydrolysis. The non-enzymatic hydrolysis at this *p*H was nil. The mixture was extracted with ether and the ether was evaporated, leading to recovered starting ester, 0.932 g. (3 mmoles), 98% yield, α_{obsd} 0.00°, 7.2% in chloroform. The infrared spectrum was identical with that of the starting ester. The

(25) G. W. K. Cavill and D. H. Solomon, *J. Chem. Soc.*, 4426 (1955).
(26) R. P. Linstead, L. N. Owen and R. F. Webb, *ibid.*, 1211 (1953).
(27) K. Serck-Hanssen, *Arkiv. Kemi*, **10**, 135 (1956).
(28) R. P. Eustigneeva, R. S. Livshitz, M. S. Bainova, L. I. Zakharbin, and N. A. Preobrachenskii, *Zhur. Obschchei Khim.*, **22**, 1467 (1952); *Chem. Abstr.*, **47**, 5949 (1953).
(29) C. R. Hauser and D. S. Breslow, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 408.

aqueous reaction solution was brought to *p*H 2, taken to dryness *in vacuo* and extracted with ether, leading to an oil residue, β-acetoxy-β-phenylpropionic acid, 0.212 g. (1.0 mmole), 84% yield, α_{obsd} 0.00°, 3.1% in chloroform. A portion of this (0.165 g., 0.80 mmole) in 10 ml. of ether was added to 0.224 g. of 1,3-bis-(*p*-dimethylaminophenyl)-carbodiimide in 15 ml. of ether, boiled under reflux for 3 hours, and cooled, leading to **1-(3-phenyl-3-acetoxypropionyl)-1,3-bis-(*p*-dimethylaminophenyl)-urea**, 0.342 g. (0.70 mmole), 88% yield, α_{obsd} 0.00°, 1.8% in chloroform, m.p. 178–179° from ether.

Anal. Calcd. for $C_{28}H_{32}O_4N_4$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.40; H, 6.73; N, 11.81.

COMMUNICATIONS TO THE EDITOR

HOMOGENEOUS HYDROGENATIONS WITH PLATINUM–TIN CHLORIDE COMPLEXES

Sir:

Although several metal ions and complexes that catalyze the homogeneous hydrogenation of unsaturated compounds have been described,[1] none is reported to be effective for ethylene[2] and acetylene. We wish to report the facile homogeneous hydrogenation of these two compounds at room temperature and atmospheric pressure with a complex platinum–tin chloride catalyst. This complex was shown earlier to catalyze the carbonylation of a variety of unsaturated compounds.[3]

A typical catalyst solution was prepared (with exclusion of oxygen) by dissolving stannous chloride dihydrate (10.0 mmoles) and chloroplatinic acid (1.0 mmole) in 120 ml. of methanol. When a 1:1 mixture of ethylene and hydrogen at a pressure of 1 atm. was admitted to the stirred catalyst solution, rapid and essentially quantitative hydrogenation occurred. Similarly, a 1:1 mixture of acetylene and hydrogen gave ethane and ethylene in about a 3:1 molar ratio.

Preliminary studies indicate that the hydrogenation of ethylene is first order in platinum. At constant platinum concentration, maximum rates are observed at tin:platinum molar ratios above about 5:1.

Pertinent to the catalytic activity of the complex is the ability of stannous chloride to promote the coordination of ethylene to platinum. Thus, reaction of ethylene at atmospheric pressure with a solution of K_2PtCl_4 containing 5 mole % of stannous chloride in 1.5 *M* HCl gave a quantitative conversion to Zeise's salt, $KPtCl_3C_2H_4 \cdot H_2O$, within 1.5 hr. In the absence of stannous chloride, no Zeise's salt formed in 16 hr. Furthermore, solutions of stannous chloride and chloroplatinic acid (6:1) in CH_3OD containing an excess of ethylene show a single n.m.r. absorption attributable to ethylenic protons that is appreciably shifted downfield compared to the absorption for ethylene in CH_3OD. This suggests an extremely rapid exchange between coördinated and free ethylene in the platinum–tin system. Since higher olefins are more difficult to hydrogenate with the complex catalyst, it appears that the ease of hydrogenation parallels the ability of the olefin to complex with platinum.

A second function of the stannous chloride is to stabilize the platinum against reduction to metal by hydrogen. Used catalyst solutions were shown to be devoid of colloidal particles by a variety of optical methods. This is in marked contrast to the behavior of simple platinum salts.

To better understand their catalytic activity, we are also examining the chemistry of the platinum–tin chloride complexes. Meyer and Ayres[4] suggested that the predominant species formed in 3 *M* hydrochloric acid is a cationic complex, $Pt(Sn_4Cl_4)^{+4}$, whereas Shukla[5] demonstrated the presence of anionic species in both the platinum–tin and rhodium–tin systems in dilute hydrochloric acid by electrophoresis. In the ruthenium–tin system, the precipitation of complexes with cations and anions was recently reported by Okuno, *et al.*[6] In none of these studies, however, were any discrete compounds fully characterized. In our work, we have established the existence of at least two complex anions and have also isolated neutral complexes as their triphenylphosphine derivatives.[7]

Addition of methyltriphenylphosphonium chloride to a deep red solution of chloroplatinic acid and stannous chloride (molar ratio 1:6) in methanol gave a quantitative yield of red, crystalline $[(C_6H_5)_3PCH_3]_3[Pt(SnCl_3)_5]$ (*Anal.* Calcd.: Cl, 24.7; Pt, 9.1; Sn, 27.6. Found: Cl, 24.2; Pt, 9.0; Sn, 27.5), soluble in acetone and nitromethane. Evaporation of the filtrate gave one mole of $[(C_6H_5)_3PCH_3]_2SnCl_6$[8] for each mole of Pt(IV) used, indicating that no reduction below Pt(II) occurs. The same red compound was obtained by reaction of phosphonium chloride, stannous chloride and K_2PtCl_4 in a molar ratio of 3:5:1 in 3 *M* hydrochloric acid.

From similar reactions at Sn:Pt of about 2:1, we have isolated yellow, crystalline $[(C_6H_5)_3PCH_3]_2[PtCl_2(SnCl_3)_2]$ (*Anal.* Calcd.: Cl, 22.4; Pt, 15.4; Sn, 18.5. Found: Cl, 22.6; Pt, 15.0; Sn, 18.8). This compound was also prepared by treating a suspension of $[(C_6H_5)_3PCH_3]_2PtCl_4$[9] in acetone with two molar equivalents of stannous chloride.

Addition of triphenylphosphine in ethyl alcohol to a solution of K_2PtCl_4 and stannous chloride (molar ratio 1:10) in 3 *M* HCl gave orange $[(C_6H_5)_3P]_2[Pt(SnCl_3)_2]$ (*Anal.* Calcd.: Cl, 18.2. Found: Cl, 18.1.) Solutions of this compound in acetone rapidly precipitated $[(C_6H_5)_3P]_2PtCl_2$. However, dissolution of $[(C_6H_5)_3P]_2PtCl_2$ in an acetone solution of stannous chloride afforded pale yellow, crystalline $[(C_6H_5)_3P]_2[PtCl(SnCl_3)]$ (*Anal.* Calcd.: Cl, 14.5; Pt, 19.9. Found: Cl, 14.8; Pt, 19.4). Both the red and yellow anionic complexes also reacted with triphenylphosphine in acetone to give $[(C_6H_5)_3P]_2PtCl_2$.

(1) See, for example, (a) J. Kwiatek, I. L. Mador and J. K. Seyler, *J. Am. Chem. Soc.*, **84**, 304 (1962); (b) J. Halpern, J. F. Harrod and B. A. James, *ibid.*, **83**, 753 (1961); (c) M. F. Sloan, A. S. Matlack and D. S. Breslow, paper presented at the Delaware Science Symposium, University of Delaware, Newark, Delaware, February 23, 1963, abstracted in the DEL-CHEM Bulletin, **19** (5), 24 (1963).

(2) The data presented by J. H. Flynn and H. M. Hulburt, *J. Am. Chem. Soc.*, **76**, 3393 (1954), may, however, be interpreted as a homogeneous hydrogenation of ethylene in the presence of $(PtCl_2C_2H_4)_2$ at temperatures below −10°.

(3) E. L. Jenner and R. V. Lindsey, Jr., U.S. Patent 2,876,254, March 3, 1959.

(4) A. S. Meyer, Jr., and G. H. Ayres, *J. Am. Chem. Soc.*, **77**, 2671 (1955).

(5) S. K. Shukla, *Ann. Chim.* (Paris), [13] **6**, 1432 (1961).

(6) H. Okuno, H. Yamatera, T. Ishemoric and K. Mezumachi, Abstract 4E3, 7th International Conference on Coördination Chemistry, 1962.

(7) Similar results have been obtained by A. G. Davies, G. Wilkinson and J. F. Young, *J. Am. Chem. Soc.*, **85**, 1692 (1963).

(8) Identical with a sample prepared from $(C_6H_5)_3PCH_3Cl$ and $SnCl_4$ in 3 *M* HCl, m.p. 294° after recrystallization from methanol–3 *M* HCl (*Anal.* Calcd.: Cl, 24.1; Sn, 13.4. Found: Cl, 24.4; Sn, 13.8).

(9) A. Michaelis and K. v. Soden, *Ann. Chem.*, **229**, 310 (1885).