General Procedure for Preparation of α-Amino Ketone Hydrochlorides (7, R′ = H) from Oxazole Compounds (4) (Step B).—After a mixture of the oxazole compounds (**4**, 0.01 mol) and 6 *N* hydrochloric acid (30 ml) was heated at 95–100° for 5 hr, the solution was washed with benzene, treated with activated charcoal, and then evaporated *in vacuo*. The obtainable crystals were washed with ethyl acetate and collected by filtration. Recrystallization from a mixture of ethyl acetate and methanol afforded various α-amino ketone hydrochlorides (**7a–k**, R′ = H) in good yields as listed in Table IV. The physicochemical properties of the resulting products in this way agreed with those obtained by step A.

Acknowledgment.—We wish to express our thanks to Drs. T. Takayanagi and I. Chibata for their encouragement in this study.

Registry No.—**1** (R′ = H; R″ = Me), 105-34-0; **1** (R′ = H; R″ = Et), 105-56-6; **3** (R = Me), 108-24-7; **3** (R = Pr), 106-31-0; **3** (R = *i*-Bu), 1468-39-9; **3** (R = CH_2Ph), 1555-80-2; **3** (R = Ph), 93-97-0; **4a**, 41172-57-0; **4b**, 41172-58-1; **4c**, 41172-59-2; **4d**, 32998-96-2; **4e**, 41172-61-6; **4f**, 41172-62-7; **4g**, 38061-18-6; **4h**, 38061-19-7; **4i**, 38061-20-0; **4j**, 38061-21-2; **4k**, 38061-22-2; **5** (R = Ph; R′ = Me; R″ = Me), 41172-68-3; **5** (R = 3,4-methylenedioxy Ph; R′ = Me; R″ = Me), 41265-39-8; **5** (R = 3,4,5-trimethoxy Ph; R′ = Me; R″ = Me), 41172-69-4; **5** (R = Ph; R′ = Et; R″ = Me), 41172-70-7; **5** (R = 3,4-methylenedioxy Ph; R′ = Et; R″ = Me), 41172-71-8; **5** (R = 3,4,5-trimethoxy Ph; R′ = Et, R″ = Me), 41172-72-9; **5** (R = Ph; R′ = *i*-Pr; R″ = Me), 41172-73-0; **5** (R = 3,4-methylenedioxy Ph; R′ = *i*-Pr; R″ = Me), 41172-74-1; **5** (R = 3,4,5-trimethoxy Ph; R′ = *i*-Pr; R″ = Me), 41172-75-2; **5** (R = 2-naphthalene; R′ = *i*-Pr; R″ = Me), 41172-76-3; **6a**, 41172-77-4; **6b**, 41172-78-5; **6c**, 41172-79-6; **6d**, 38603-81-5; **6e**, 6317-41-5; **6f**, 41172-82-1; **6g**, 38061-23-3; **6h**, 38061-24-4; **6i**, 38061-25-5; **6j**, 38061-26-6; **6k**, 38061-27-7; **6l**, 40846-67-1; **6m**, 38061-28-8; **6n**, 38061-29-9; **6o**, 40846-68-2; **6p**, 40846-71-7; **6q**, 40846-74-0; **6r**, 38061-30-2; **6s**, 38061-31-3; **6t**, 40846-73-9; **6u**, 38061-32-4; **7a**, 7737-17-9; **7b**, 41172-98-9; **7c**, 21419-26-1; **7d**, 41173-00-6; **7g**, 5468-37-1; **7h**, 38061-34-6; **7i**, 38061-35-7; **7k**, 38061-36-8; **7m**, 38061-37-9; **7n**, 38061-38-0; **7r**, 33119-73-2; **7t**, 38061-40-4; succinic anhydride, 108-30-5; glutaric anhydride, 108-55-4; DBU, 6674-22-2.

Selective Hydrolysis of Dihydrocinnamate Ester Protecting Groups by α-Chymotrypsin. Further Studies on the Scope and Limitations of the Method

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Further examples of the preparative feasibility of exploiting the aromatic acyl group specificity of α-chymotrypsin as a useful addition to the methods of protecting group chemistry have been provided by studies on representative decalin and tropane acetate–dihydrocinnamate diesters. When the hydroxide-ion susceptibility of both ester functions is approximately equal, α-chymotrypsin catalysis exposes only the hydroxyl group protected as its dihydrocinnamate ester. On the other hand, when hydroxide-ion selectivity of hydrolysis is possible, enzymic hydrolysis of the dihydrocinnamate function can be used to complement or reverse the chemical specificity. The alcohol moiety binding site of the enzyme is hydrophobic in character and tolerates wide structural variations and the method appears applicable to a broad range of organic compounds provided that a marginal solubility in aqueous solutions is preserved. The approach seems most appropriate for alkaloids where partial conjugate acid formation can enhance the water solubility of the substrate. The mild, pH 7.8, conditions used are synthetically attractive since they minimize the problems of epimerization, isomerization, racemization, rearrangement, etc., often encountered during acid- or base-mediated hydrolyses of ester protecting groups.

In view of the increasing availability of purified enzymes and their immobilized derivatives, there can be little doubt that preparative exploitations of enzymes as selective and/or stereospecific catalysts of organic reactions will become far more widespread in the future. One of the areas in which the synthetic advantages of enzymatic catalysis has already been recognized is in the use of α-chymotrypsin to effect selective removal of aromatic amino acid and related acyl protecting groups from hydroxyl and amino functions of nucleosides.[1–4] Our preliminary studies[5] indicated that the method might be generally applicable and this communication provides further data on the scope and limitations of the technique when extended to mixed esters of other classes of organic compounds.

Choice and Syntheses of Some Representative Diols and their Esters.—In our initial survey,[5] it was found that, whereas simple acetate and dihydrocinnamate esters were hydrolyzed at almost equivalent rates by hydroxide ion,[6] α-chymotrypsin-catalyzed cleavage of the dihydrocinnamoyl moiety was achieved with complete specificity with acyclic acetate–dihydrocinnamate diesters. Accordingly, mixed diesters of this type were used to evaluate the preparative selectivity of α-chymotrypsin toward the dihydrocinnamoyl group in molecules of more general structural interest to organic chemists.

It was desired to evaluate the feasibility of achieving preferential removal of the α-chymotrypsin-sensitive acyl group from acetate dihydrocinnamates for which differences in the rates of chemical hydrolysis of each ester function were small or nonexistent. Alternatively, for compounds where preferential chemical cleavage of one ester group was possible, exploitation of α-chymotrypsin's specificity to reverse the chemical selectivity was the goal. Mixed esters of *trans*-decalin-1,6-diols and of tropane-3α,6β-diol appeared to satisfy most of the requirements for such evaluation and selected mixed ester derivatives were prepared as described below.

(1) Z. A. Shabarova, N. I. Sokolova, and M. A. Prokov'ev, *Dokl. Akad. Nauk SSSR*, **128**, 740 (1959).

(2) N. I. Sokolova, L. A. Borkova, and M. A. Prokov'ev, *Zh. Obshch. Khim.*, **29**, 2917 (1959).

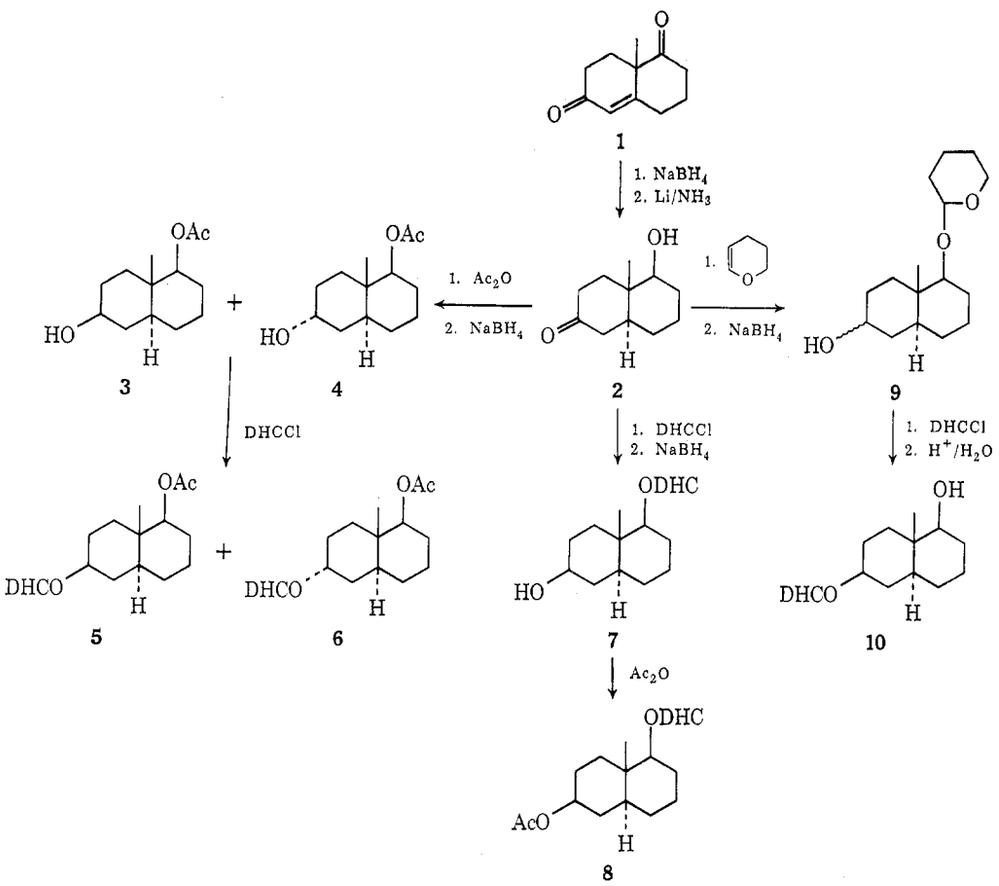
(3) D. H. Rammler and H. G. Khorana, *J. Amer. Chem. Soc.*, **85**, 1997 (1963).

(4) H. S. Sachdev and N. A. Starkovsky, *Tetrahedron Lett.*, 733 (1969); A. Taunton-Rigby, *J. Org. Chem.*, **38**, 977 (1973).

(5) J. B. Jones and Y. Y. Lin, *Can. J. Chem.*, **50**, 2053 (1972).

(6) M. L. Bender, F. J. Keźdy, and C. R. Gunter, *J. Amer. Chem. Soc.*, **86**, 3714 (1964).

SCHEME I



The synthetic pathways leading to the desired *trans*-decalin esters are outlined in Scheme I.[7] Sodium borohydride reduction of 9-methyl-$\Delta^{5(10)}$-octalin-1,6-dione (**1**) followed by lithium in liquid ammonia treatment of the resulting 1β-alcohol gave the 1β-hydroxy-6-ketone **2** in good yield.[8] 1β Acetylation of **2** followed by a second sodium borohydride reduction proceeded smoothly to yield a difficult-to-separate mixture of the C-6 epimeric alcohols **3** and **4** in which glc analysis showed the equatorial 6β-hydroxy compound to predominate to the extent of ~85%. Esterification of the mixture of **3** and **4** with dihydrocinnamoyl chloride afforded the corresponding mixture of diesters **5** and **6** from which the 6β epimer **5** was fairly readily isolable. In contrast, exhaustive chromatography was required before a small sample of the pure 6α-dihydrocinnamate **6** could be obtained.[9] The 6β-acetoxy-1β-dihydrocinamate **8** was obtained from **2** in a similar manner by reversing the order of the esterification steps. Conversion of **2** into the 6β-dihydrocinnamate **10**, while retaining the β-hydroxyl function at C-1, was accomplished *via* the 1β-tetrahydropyranyloxy derivative **9**. The C-1 and C-6 configurational assignments of the compounds prepared were confirmed by the characteristic shape and relative chemical shifts of the 1α- and 6α- and β-proton peaks in the nmr spectra.[10]

The synthetic steps carried out in the tropane series are summarized in Scheme II. 6β-Hydroxytropinone (**11**)[11] was stereospecifically reduced[12] to the 3α,6β-diol **12**, which was then esterified with dihydrocinnamoyl chloride to give the bisdihydrocinnamate **13**. Selective[13] hydrolysis of the 6β-ester function of **13** proceeded smoothly and the 3α-dihydrocinnamate **14** obtained was converted into the mixed diester **15** by treatment with acetic anhydride. Conversion of 6β-hydroxytropinone (**11**) into the 6β-acetoxy ketone **16** followed by hydrogenation over Raney nickel to the 3α-hydoxy-6β-acetate **17** and then treatment with dihydrocinnamoyl chloride provided a convenient alternative synthesis of **15**. The C-3 and C-6 configurations assigned[14] to the compounds of Scheme II were supported by the chemical shifts and multiplicities observed for the protons at these positions. In the spectra of the 3-oxotropanes **11** and **16** the C-6α protons appeared as triplets. However, when 3α-hydroxy or ester functions were present, the multiplicity of this peak changed to a doublet of doublets. In contrast, the C-3β protons were consistently observed as triplets.

(7) In all schemes, the dihydrocinnamoyl function is represented as DHC.

(8) A. J. Birch, E. Pride, and H. Smith, *J. Chem. Soc.*, 4688 (1958).

(9) Similar separation difficulties were encountered during all subsequent attempts to obtain pure samples of each of the minor (<15% in each case) 6α axially substituted derivatives encountered in Scheme I. Accordingly, all work carried out following the separation of **5** and **6** was concentrated on the predominant and more readily purified 6β-equatorial compounds.

(10) R. U. Lemieux, R. K. Kulling, H. J. Bernstein, and W. G. Schneider, *J. Amer. Chem. Soc.*, **80**, 6098 (1958).

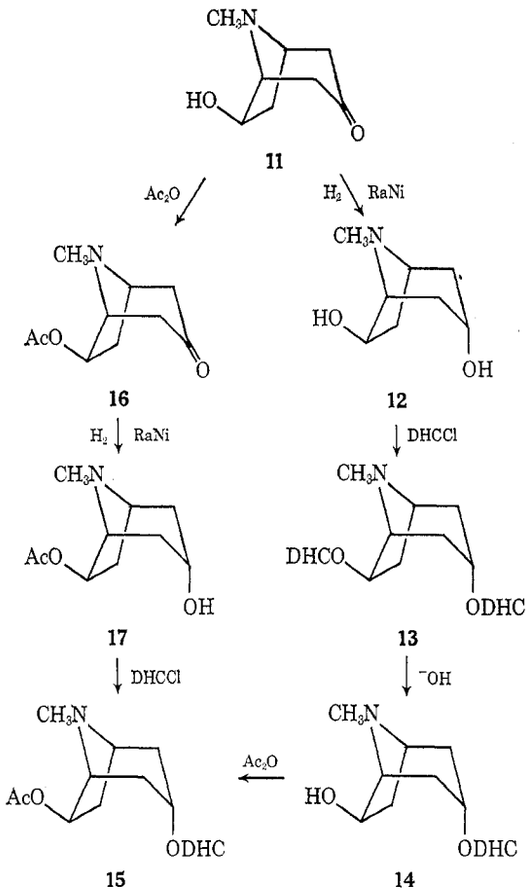
(11) P. Nedenskov and N. Clauson-Kaas, *Acta Chem. Scand.*, **8**, 1295 (1954).

(12) A. Stoll, B. Becker, and E. Jacker, *Helv. Chim. Acta*, **35**, 1263 (1952).

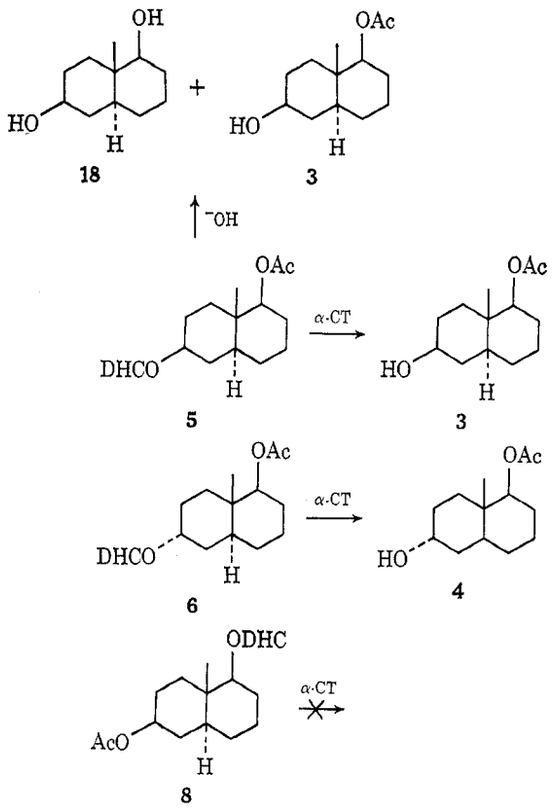
(13) G. Folder, I. W. Vinege, and J. Toth, *J. Chem. Soc.*, 3219 (1961); G. Folder, S. Kiss, and J. Rakoczi, *Chem. Ind. (London)*, **90**, 225 (1963).

(14) G. Fodor in "Chemistry of the Alkaloids," S. W. Pelletier, Ed., Van Nostrand-Reinhold, Princeton, N. J., 1970, p 449.

SCHEME II



SCHEME III



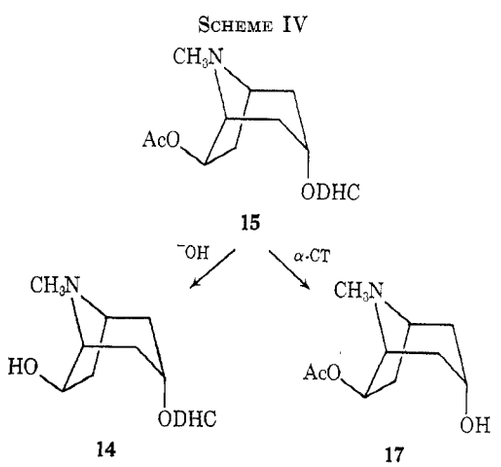
Results

On the basis of literature data on the hydrolysis of *trans*-decalin[15] and analogous steroid[16] diesters, some preferential hydrolysis of the 6β function of the diester **5** by hydroxide ion was expected. However, that this degree of chemical selectivity was of limited preparative utility on a routine basis was demonstrated by the product distribution observed when **5** was treated with 1 equiv of sodium hydroxide in aqueous methanol at 25°. Only 53% of the 1β-acetate **3** was isolated, and furthermore, 35% of the 1β,6β-diol product **18** of complete hydrolysis was also formed. In contrast, α-chymotrypsin-catalyzed hydrolysis of **5** resulted in completely specific removal of the 6β-dihydrocinnamoyl group, and quantitative conversion into the 1β-acetate **3** was achieved (Scheme III) with none of the 6β-dihydrocinnamoyloxy-1β-alcohol **10** being formed. Although the rate of hydrolysis of the epimeric 6α-dihydrocinnamate **6** was significantly slower, its selective enzymic conversion into the 6α-hydroxy-1β-acetate **4** also occurred to the extent of 96%. For the isomeric, but much less soluble, 1β-dihydrocinnamate-6β-acetate diester **8**, the enzyme-mediated reaction was much more sluggish than for **5** or **6** and α-chymotrypsin-catalyzed hydrolysis was not preparatively feasible for this compound.

On treatment of diesters, such as the acetates or isovalerates, of 3α,6β-dihydroxytropane with hydroxide ion under controlled conditions, hydrolysis of only the 6β function can be achieved.[18] The mixed 6β-acetate–3α-dihydrocinnamate diester **15** was therefore synthesized in order to evaluate the ability of α-chymotrypsin to reverse the hydroxide ion selectivity by effecting specific cleavage at the 3α position.

The results obtained are represented in Scheme IV.

SCHEME IV



That selective hydroxide ion mediated cleavage of the 6β-ester function could be effected was confirmed by the hydrolysis of **15** and of the bisdihydrocinnamate **13** to the 6β-hydroxy-3α-cinnamate **14** in 65–70% yields. The hydrochloride salt of **15** was used as the substrate for the α-chymotrypsin experiments, and consequently, addition of ~0.5 equiv of sodium hydroxide was required in order to bring the reaction solution up to the pH optimum of 7.8 prior to the addition of the enzyme. When the enzyme-catalyzed

(15) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," Interscience, New York, N. Y., 1965, p 237.

(16) L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, N. Y., 1959, p 513.

hydrolysis ceased, a further 0.5 equiv of base had been consumed and the desired product of 3α hydrolysis, 6β-acetoxy-3α-hydroxytropane (**17**), was obtained in quantitative yield.[17]

The relative rates of hydrolysis under preparative conditions of all the compounds studied, and, where relevant, the degree of selectivity of the desired α-chymotrypsin-catalyzed cleavage, are summarized in Table I. For **15** and the two most soluble dihydrocinnamate esters of the decalin series, **7** and **10**, quantitative kinetic data were obtainable under homogeneous conditions. The kinetic parameters obtained are recorded in Table II.

TABLE I

PRODUCT ANALYSES AND RELATIVE RATE DATA FOR PREPARATIVE-SCALE α-CHYMOTRYPSIN-CATALYZED HYDROLYSES OF DIHYDROCINNAMATE ESTERS[a]

Compd	Product(s) (% selectivity)[b]	Relative hydrolysis rates[c]
$C_6H_5(CH_2)_2COOCH_3$		1.0
5	**3** (100)	0.51
6	**4** (96)	0.11
8[d]		0.03
7	**18**	0.16
10	**18**	2.02
15	**17** (100)	0.49

[a] The preparative-scale enzyme-catalyzed hydrolyses were carried out on heterogeneous suspensions of up to 2 g of the substrates in 0.1 *M* aqueous KCl at 25° and pH 7.8. Concentrations of α-chymotrypsin of 1 mg/ml of reaction volume were used. [b] By glc analysis; each product was also isolated and characterized. [c] Determined from initial velocities of hydrolyses carried out under standardized conditions in solutions containing 5% acetonitrile. [d] Too slow to be preparatively viable.

TABLE II

KINETIC DATA FOR THE α-CHYMOTRYPSIN-CATALYZED HYDROLYSES OF THE DIHYDROCINNAMATE ESTERS **7**, **10**, AND **15**[a]

Substrate	k_{cat}, sec^{-1}	K_m, $M \times 10^3$	k_{cat}/K_m, $M^{-1}\ sec^{-1}$
$C_6H_5(CH_2)_2COOCH_3$	0.13	8.46	15.4
7[b]	0.009	0.4	22.5
10[b]	0.085	0.7	121.4
15	0.09	2.95	30.5

[a] Kinetic determinations were carried out with a pH-Stat at 25° at pH 7.8 in aqueous 0.1 *M* KCl solutions containing 20% acetonitrile for all substrates except **15**. For the latter, no organic cosolvent was required. [b] Under the same conditions k_{OH} ($M^{-1}\ sec^{-1} \times 10^3$) values for **7** and **10** are 0.7 and 1.6, respectively.

The overall data obtained show that the structure of the alcohol moiety, and also the solubility of the overall molecule in aqueous solutions, have a considerable influence on the hydrolysis process.

Discussion

All of the α-chymotrypsin-catalyzed hydrolyses were carried out at the pH 7.8 optimum of the enzyme. At this pH, no nonenzymic hydrolysis of the ester groups could be detected. Since the diesters evaluated were of limited solubility in water, the reactions were carried out on heterogeneous mixtures. For the most hydrophobic and more slowly hydrolyzed compounds, such as **5**, **6**, and **8**, a small percentage of acetonitrile was sometimes added to increase the water solubility of the potential substrate. However, owing to the inactivation of the enzyme which occurs on addition of an organic cosolvent of this type,[18] the acetonitrile content was kept below 5% for the preparative-scale experiments. The rates of hydrolyses were also significantly reduced when other aprotic solvents, such as tetrahydrofuran and dioxane, were used to increase the solubility of the more hydrophobic substrates. The adverse influence of dimethyl sulfoxide on the enzyme was less marked,[19] but, unfortunately, its effect on the water solubility of the decalin substrates was small. Attempted hydrolyses in the presence of the nonionic surfactants Tween-80 and Brij-35, and in biphasic benzene–water systems, were also unsatisfactory. In fact, in spite of the heterogeneous nature of most of the reaction mixtures, the most rapid hydrolyses were observed when no cosolvent or other additives were present. As the reactions proceeded and the extent of dihydrocinnamate cleavage increased, the solutions often became homogeneous. The pH was maintained at 7.8 by pH-Stat-controlled[20] addition of aqueous sodium hydroxide. For the better substrates, such as **5** and **15**, the hydrolysis virtually ceased soon after the uptake of 1 equiv of base. However, owing to slow autolysis of the enzyme during the reaction periods (up to 24 hours) of the other substrates such as **6**, the addition of 1 equiv of sodium hydroxide did not always signify the end of the desired selective cleavage. In such cases, some starting material was recovered. Concentrations of enzyme of 1 mg/ml were generally found to be the most satisfactory. At this level, the activity of the enzyme remained relatively constant for up to 24 hr at 25°. The desired products of selective hydrolysis were very readily isolated by simple extraction and chromatographic procedures. Dihydrocinnamic acid was also isolable from the acidified aqueous solutions.

The *trans*-decalin acetate–dihydrocinnamates **5**, **6**, and **8** were selected as representative diesters for which the differences in the rates of hydroxide attack of the different acyl functions were unlikely to be large enough to be generally exploitable for preparative purposes.[15] The validity of this supposition was confirmed by the relative lack of selectivity exhibited during the hydroxide ion hydrolysis of **5**. In contrast, α-chymotrypsin-catalyzed hydrolyses of the dihydrocinnamate functions of **5** and **6** were completely stereospecific and the desired monoacetates **3** and **4**, respectively, were produced to the extent of $\geq 96\%$. Unfortunately, no significant enzymic catalysis could be demonstrated for the isomeric acetate, dihydrocinnamate **8**. The extreme insolubility of this compound in the aqueous reaction media was considered one of the major factors responsible for the inability of α chymotrypsin to catalyze its hydrolysis. A semi-

(17) A small proportion of **17** was isolated as its dihydrocinnamate salt. However, this was readily reconverted to the free base with aqueous potassium carbonate.

(18) G. E. Clement and M. L. Bender, *Biochemistry*, **2**, 836 (1963).

(19) U. I. Khurgin, V. Y. Koslyakov, U. M. Azizov, and E. D. Kaverzneva, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 2840 (1968).

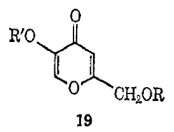
(20) As described previously,[5] addition of base from a buret is also satisfactory for good substrates. Use of buffered solutions should also be satisfactory.

quantitative evaluation of the adverse influence on the hydrolysis rate of the low water solubility of the hydrophobic decalin diesters was provided by a comparison (Table I) of the ease of dihydrocinnamate cleavage from **5** and **8** with those of their hydroxy analogs **10** and **7**, respectively. The rates of enzymic hydrolysis of the latter more hydrophilic substrates are four to five times greater than those of the corresponding diesters. Addition of the organic cosolvents acetonitrile, tetrahydrofuran, dioxane, and dimethyl sulfoxide to increase the water solubility of the hydrophobic decalin substrates was somewhat self-defeating, since aprotic dipolar solvents are believed to compete with substrate binding at the active site.[18]

As indicated above, selective hydroxide-ion cleavage is unlikely to be of much preparative value for decalin diesters such as **5**, **6**, and **8**. However, for di- or polyesters where one or more functions can be selectively cleaved in this way, the use of an appropriate combination or sequence of chemical and enzymic hydrolysis could be advantageous in a synthetic sequence using ester protecting groups. The controlled hydrolysis of 3α,6β-dihydroxytropane diesters was used to exemplify this type of application.

The starting material used, 6β-hydroxytropinone (**11**), permits two illustrations of how the method can be applied to a diol or a keto alcohol. Esterification of the diol **12** with dihydrocinnamoyl chloride gave the 3α,6β-bisdihydrocinnamate **13**. This, on treatment with 1 equiv of sodium hydroxide under controlled conditions, afforded 3α-dihydrocinnamoyl-6β-hydroxytropane (**14**) in 65% yield. Reprotection of the 6β-hydroxyl group as the α-chymotrypsin-insensitive acetate gave the mixed diester **15**. On being subjected to enzymic hydrolysis conditions, **15** underwent specific dihydrocinnamate hydrolysis at the chemically less accessible 3α position to give the 6β-acetoxy-3α-alcohol **17** in quantitative yield. Alternatively, starting from a compound of mixed functionalization such as **11**, the 6β-alcohol can be protected directly as the acetate, and the 3-ketone then transformed into the 3α-dihydrocinnamate. The mixed diester **15** so obtained could then be selectively hydrolyzed (Scheme IV) by hydroxide ion to give the 3α-dihydrocinnamate-6β-ol **14** in 70% yield or specific and quantitative 3α cleavage could be induced by α-chymotrypsin as described above.[21]

(21) All of the esters discussed in this communication were unaffected by pH 7.8 aqueous conditions in the absence of enzyme. In order to provide a more severe test of the scope of the method with respect to more sensitive esters, enol and allylic ester derivatives of kojic acid (**19**, R = R′ = H), for



19

which piperidine-catalyzed chemical hydrolysis at the 5 position is possible,[22] were evaluated. The desired selective hydrolysis of the bisdihydrocinnamate **19**, R = R′ = DHC, was readily achieved and the allylic dihydrocinnamate **19**, R = DHC, R, = H, obtained was reesterified with acetic anhydride. Disappointingly, it was found that, at pH 7.8, hydrolysis of *both* ester functions of **19**, R = DHC, R′ = Ac, were facile and that, although addition of α-chymotrypsin accelerated the reaction, the susceptibility of the enol acetate function to hydrolysis overwhelmed the DHC specificity of the enzyme. No reversal of the chemical selectivity was achievable within the pH range 4–9 surveyed. Use of aromatic amino acid acyl protecting groups for which the α-chymotrypsin specificity is much greater[2,3] might help to surmount this problem.

(22) M. G. Brown, *J. Chem. Soc.*, 2558 (1956).

As would have been predicted,[23] the more sterically hindered of the dihydrocinnamate esters are hydrolyzed the least rapidly by α-chymotrypsin (Table I). Although this is also true for the corresponding hydroxide-ion cleavages, the enzyme-catalyzed reactions generally expand the rate differences. For example, the rate constants for hydroxide hydrolysis of the 6β-dihydrocinnamate **10** and 1β-dihydrocinnamate **7** differ by a factor of 2:1 (Table II). In contrast, for the α-chymotrypic cleavages of the same esters, an ~13-fold rate difference is observed. Not unexpectedly, variations in the relative rates of hydroxide and enzymic hydrolyses of axial and equatorial esters are less dramatic. The 3:1 ratio of hydrolysis rates expeced[24] for the 6β- and 6α-dihydrocinnamates **5** and **6** is somewhat enhanced by α-chymotrypsin with the 6β-equatorial epimer **5** being cleaved five times faster than **6**.

Further analysis of the data of Table I, and particularly of Table II, enables more quantitative deductions to be drawn regarding the influence of the alcohol structure on the enzyme-catalyzed hydrolysis and on the preparative viability of such selective dihydrocinnamate cleavages. For methyl dihydrocinnamate the overall rate constant k_{cat} is markedly influenced by the acylation rate constant k_2,[25] since the latter is significantly smaller than that for deacylation, k_3. The lower k_{cat} values (Table II) for **7**, **10**, and **15** are attributed to a further slowing of the acylation rate relative to methyl dihydrocinnamate[27] owing to less favorable orientations of the bulky ester moieties at the active site. On the other hand, the decreased K_m values (compared with methyl dihydrocinnamate) of **7** and **10** show that the hydrophobic decalin moieties contribute significantly to binding[28] in the ES complex. This further evidence that the alcohol binding area is hydrophobic in character[23,29] is supported by the higher (relative to **7** and **10**) K_m observed for **15**.[30] Under the assay conditions the tropane residue of this substrate is partially protonated and is thus quite hydrophilic in character. The specificity constant

(23) P. W. Inward and W. P. Jencks, *J. Biol. Chem.*, **240**, 1986 (1965).

(24) By analogy with structurally similar steroid esters studied by L. Ruzicka, M. Furter and M. W. Goldberg, *Helv. Chim. Acta*, **21**, 468 (1938).

(25) The usual[26] α-chymotrypsin steady-state kinetics terminology has been used as outlined below.

$$E + S \underset{}{\overset{K_s}{\rightleftharpoons}} ES \xrightarrow[\text{acylation}]{k_2} ES' \xrightarrow[\text{deacylation}]{k_3} E + P$$

For such situations $k_{cat} = k_2k_3/k_2 + k_3$ and $K_m = k_3/K_s/k_2 + k_3$.

(26) M. L. Bender, "Mechanisms of Homogeneous Catalysis from Protons to Proteins," Wiley-Interscience, New York, N. Y., 1971, p 494.

(27) M. L. Bender, F. J. Kézdy, and C. R. Gunter, *J. Amer. Chem. Soc.*, **86**, 3714 (1964).

(28) While the lower [relative to $C_6H_5(CH_2)_2COOCH_3$] k_2 values of **7** and **10** are partly responsible for the lower K_m's observed, it is unlikely that the k_2 changes are large enough to account completely for the >tenfold K_m reductions observed for these compounds. Thus, while the K_m values of Table II are not equal to K_s, they are considered to reflect the strength of binding in the ES complex. However, the fact that the substrates evaluated are racemates must be borne in mind, since the limited data available[29] indicate that significant stereospecificity is possible for interactions of enantiomeric alcohol moieties with the active site.

(29) N. S. Isaacs and C. Niemann, *Biochim. Biophys. Acta*, **44**, 196 (1960); B. Halpern, J. Ricks, and J. W. Westley, *Aust. J. Chem.*, **20**, 389 (1967). Additional data on this aspect have been obtained: J. B. Jones, Y. Y. Lin, and D. N. Palmer, *Can. J. Chem.* in press.

(30) The tropane diester **15** was soluble enough in aqueous solutions to enable the kinetic constants to be obtained without the addition of the acetonitrile. Lower k_{cat} and higher K_m constants would be anticipated[31] for **15** if measurements had been made under the 20% acetonitrile conditions required for the more hydrophobic substrates of Table II.

(31) T. H. Applewhite, R. B. Martin, and C. Niemann, *J. Amer. Chem. Soc.*, **80**, 1457 (1958).

k_{cat}/K_m is considered the most reliable indicator of substrate activity[32] and the values recorded in Table II for **7**, **10**, and **15** correlate well with their rates of hydrolysis under preparative conditions. However, for the most hydrophobic of the substrates included in Table I, solubility factors become a very important consideration and predictions regarding preparative viability based on specificity constant data of very hydrophobic compounds must be applied with caution.

The current and previous[1-5] results demonstrate clearly that the use of α-chymotrypsin to achieve selective ester hydrolysis represents a useful addition to the methods of protecting group chemistry. Furthermore, the often encountered problems of epimerization, racemization, isomerization, rearrangement, etc., should be minimal since the hydrolyses are effected under mild, neutral conditions. In view of the hydrophobic nature and broad structural tolerance of the alcohol moiety binding site of the enzyme, the method should be applicable to most classes of organic compounds, provided that a satisfactory balance of hydrophobic and hydrophilic properties is maintained.

Experimental Section[33]

1β-Hydroxy-8aβ-methyl-1,2,3,4,4aα,5,8,8a-octahydronaphthalen-6(7*H*)-one (2).—Reduction of 8aβ-methyl-3,4,8,8a-tetrahydronaphthalene-1,6(2*H*,7*H*)-dione (1) with sodium borohydride as described by Boyce and Whitehurst[34] gave the corresponding 1β alcohol, mp 49–53° (lit.[34] mp 50–55°), which on subsequent treatment with lithium in liquid ammonia according to the method of Birch, *et al.*,[8] yielded the 1β-hydroxy-6-ketone **2**, mp 67–68° (lit.[8] mp 68–70°), nmr δ 1.07 (3, s, C-8a Me) and 3.33 ppm (1, m, C-1α H).

1β-Acetoxy-6β-dihydrocinnamoyloxy-8aβ-methyl-1,2,3,4,4aα,5,6,7,8,8a-decahydronaphthalene (5) and 1β-Acetoxy-6α-dihydrocinnamoyloxy-8aβ-methyl-1,2,3,4,4aα,5,6,7,8,8a-decahydronaphthalene (6).—The 1β-hydroxy-6-ketone **2** was acetylated with acetic anhydride in pyridine[8] to give the corresponding 1β-acetoxy-6-ketone: mp 49–51° (lit.[8] mp 49–50°); nmr δ 1.13 (3, s, C-8a Me), 2.07 (3, s, $COCH_3$), and 4.57 ppm (1, m, C-1α H). The preceding keto acetate (3 g, 13.4 mmol) was reduced with sodium borohydride (300 mg, 7.9 mmol) in absolute ethanol (100 ml). Following the usual[34] work-up and chromatography on alumina (grade II) with ethyl acetate–benzene elution, 2 g of a mixture containing the 6β and 6α epimers **3** and **4** in the ratio 9:1 was obtained. Further purification by distillation, bp 170° (0.025 Torr), and exhaustive chromatography gave small samples of the 1β-acetoxy-6β-hydroxy-8aβ-methyl-*trans*-decalin **3**, nmr δ 0.92 (3, s, C-8a Me), 2.02 (3, s, $COCH_3$), 3.57 (1, m, C-6α H), and 4.53 ppm (1, m, C-1α H), and the 1β-acetoxy-6α-hydroxy-8aβ-methyl-*trans*-decalin **4**, nmr δ 4.06 ppm (1, m, C-6β H).

The mixture of 6α- and 6β-hydroxy epimers **3** and **4** (1.3 g, 5.7 mmol) in pyridine (1 ml) and dry ether (30 ml) was heated under reflux with dihydrocinnamoyl chloride (1.04 g, 6.2 mmol) for 3 hr. The reaction mixture was then diluted with ether (50 ml) and washed with dilute hydrochloric acid, then with saturated aqueous sodium bicarbonate, and the ether solution was dried ($MgSO_4$). Evaporation of the ether followed by chromatography on Florisil (ethyl acetate–benzene elution) gave the 6α-dihydrocinnamate **6** (50 mg): nmr δ 0.87 (3, s, C-8a Me), 1.93 (3 s, $COCH_3$), 2.77 (4, A_2B_2 m, $C_6H_5CH_2CH_2CO$), 4.49 (1, m, C-1α H), 4.97 (1, m, 6β-H) and 7.20 ppm (5, s, C_6H_5).

Anal. Calcd for $C_{22}H_{30}O_4$: C, 73.71; H, 8.43. Found: C, 73.84; H, 8.53.

Continued elution with the same solvent yielded the 6β-dihydrocinnamate **5** (820 mg): bp 205° (bath, 0.025 Torr); nmr δ 0.90 (3, s, C-8a Me), 1.95 (3, s, $COCH_3$), 2.76 (4, A_2B_2 m, $C_6H_5CH_2CH_2CO$), 4.51 (2, m, C-1α and C-6α H), and 7.17 ppm (5, s, C_6H_5).

Anal. Calcd for $C_{22}H_{30}O_4$: C, 73.71; H, 8.43. Found: C, 73.61; H, 8.46.

1β-Dihydrocinnamoyloxy-6β-hydroxy-8aβ-methyl-1,2,3,4,4aα,5,6,7,8,8a-decahydronaphthalene (7).—Dihydrocinnamoyl chloride (1.9 g, 11.3 mmol) and the 1β-hydroxy-6-ketone **2** (2 g, 11 mmol) were refluxed for 3 hr in dry ether (30 ml) and pyridine (2 ml). After dilution with ether, the reaction mixture was washed with dilute hydrochloric acid, then with aqueous sodium bicarbonate, and the dried ($MgSO_4$) ether solution was evaporated. The residual oil (2.3 g) was recrystallized from ether–hexane to give the 1β-dihydrocinnamoyloxy-8aβ-methyl-1,2,3,4,4aα,5,8,8a-octahydronaphthalen-6(7*H*)-one: mp 53–53°; nmr δ 1.06 (3, s, C-8a Me), 2.83 (4, A_2B_2 m, $C_6H_5CH_2CH_2CO$), 4.57 (1, m, C-1α H), and 7.26 ppm (5, s, C_6H_5).

Anal. Calcd for $C_{20}H_{26}O_3$: C, 76.39; H, 8.83. Found: C, 76.61; H, 8.40.

The above 1β-dihydrocinnamoyloxy-6-ketone (2.2 g, 7 mmol) was reduced with sodium borohydride (132 mg, 3.5 mmol) in dry ethanol (50 ml) in the usual[34] manner. The oil (2.1 g) obtained contained the 6β- and 6α-hydroxy epimers in the ratio of ~4:1. After careful chromatography of this mixture on alumina (cyclohexane–ethyl acetate elution), the 6β-hydroxy-1β-dihydrocinnamate **7** (1 g), bp 200° (0.05 Torr), was isolated: nmr δ 0.91 (3, s, C-8a Me), 2.78 (4, A_2B_2 m, $C_6H_5CH_2CH_2CO$), 3.58 (1, m, C-6α H), 4.51 (1, m, C-1α H), and 7.23 ppm (5, s, C_6H_5).

Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.91. Found: C, 75.84; H, 8.89.

6β-Acetoxy-1β-dihydrocinnamoyloxy-8aβ-methyl-1,2,3,4,4aα,5,6,7,8,8a-decahydronaphthalene (8).—The 6β-hydroxy-1β-dihydrocinnamate **7** (500 mg, 1.6 mmol) was acetylated with acetic anhydride (2 g) in pyridine (3 ml). After work-up, the reaction product was chromatographed on Florisil (benzene–ethyl acetate elution) and then further purified by tlc. The diester **8** was obtained as an oil (200 mg): nmr δ 0.87 (3, s, C-8a Me), 1.93 (3, s, $COCH_3$), 2.72 (4, A_2B_2 m, $C_6H_5CH_2CH_2CO$), 4.50 (2, m, C-1α and C-6α H), and 7.17 ppm (5, s, C_6H_5).

Anal. Calcd for $C_{22}H_{30}O_4$: C, 73.71; H, 8.43. Found: C, 73.72; H, 8.48.

6β-Dihydrocinnamoyloxy-1β-hydroxy-8aβ-methyl-1,2,3,4,4aα,5,6,7,8,8a-decahydronaphthalene (10).—To a solution of the 1β-hydroxy-6-ketone **2** (2.2 g, 12.1 mmol) in dry ether (40 ml) was added dihydropyran (3.05 g, 36.3 mmol) and phosphoryl chloride (0.2 g). The mixture was stirred at 20° for 10 min and methanolic potassium hydroxide was added until the mixture remained basic. Following dilution with ether (100 ml), the solution was washed with water, dried ($MgSO_4$), and evaporated. The oil obtained was recrystallized from petroleum ether (bp 40–60°) to give 8aβ-methyl-1β-tetrahydropyranyloxy-1,2,3,4,4aα,5,8,8a-octahydronaphthalen-6(7*H*)-one (3 g): mp 66–71°; nmr δ 1.04 (3, s, C-8a Me) and 4.63 ppm (2, m, C-1α H and THP-CH).

Anal. Calcd for $C_{16}H_{26}O_3$: C, 72.14; H, 9.83. Found: C, 72.17; H, 9.84.

The above tetrahydropyranyloxy ketone (2.9 g, 11 mmol) was reduced with sodium borohydride (126 mg, 3.1 mmol) in dry ethanol (50 ml) as before.[34] The product obtained (2.9 g) was mainly the 6β epimer of **9**, nmr δ 0.96 (3, m, C-8a Me) and 4.56 ppm (2, m, C-1α H and THP-CH).

The 6ξ-hydroxy-1β-tetrahydropyranyloxy mixture **9** (2.8 g, 10.4 mmol) in dry ether (50 ml) and pyridine (0.5 ml) was treated with dihydrocinnamoyl chloride (1.75 g, 10.4 mmol) as described previously. After chromatography of the crude product on Florisil using benzene–ether elution, 6β-dihydrocinnamoyloxy-8aβ-methyl-1β-tetrahydropyranyloxy-1,2,3,4,4aα,5,6,7,8,8a-decahydronaphthalene (2.9 g, containing ~5% of the 6α epimer) was obtained: nmr δ 0.87 (3, s, C-8a Me), 2.73 (4, A_2B_2 m, $C_6H_5CH_2CH_2CO$), 4.60 (3, m, C-1α, C-6α H, THP-CH), and 7.17 ppm (5, s, C_6H_5).

The above material (2.8 g, 7 mmol) in ethanol (20 ml) and 2 *N* HCl (4 ml) was heated at 50° for 3 hr. The solvent was then

(32) F. E. Brot and M. L. Bender, *J. Amer. Chem. Soc.*, **91**, 7187 (1969).

(33) Melting points were determined on a Fisher-Johns block and are uncorrected. The purity of all compounds was checked by glc and tlc analyses. Glc data were obtained using an F & M 400 chromatograph equipped with 1%, 2% QF-1 and 1% PDEAS columns. Tlc analyses and separations were performed on silica gel G; all solvents were redistilled before use. Ir spectra (measured in $CHCl_3$ solutions) were all as expected for the assigned structures and are not recorded. Nmr data were obtained in CCl_4 or $CDCl_3$ (unless indicated otherwise) using TMS as internal standard. Starting materials were generally purchased from Aldrich and were used as obtained unless impurities were detected by glc or tlc. α-Chymotrypsin (three times crystallized) was purchased from the Worthington Biochemical Corp. Solvent removals were effected by rotary evaporation under reduced pressure at room temperature.

(34) C. B. C. Boyce and J. S. Whitehurst, *J. Chem. Soc.*, 2680 (1960).

removed and the residue was dissolved in ether (50 ml). The ether solution was washed with aqueous sodium bicarbonate, dried ($MgSO_4$), and evaporated to give an oil (2 g) which was then chromatographed on alumina. Careful elution with ethyl acetate–cyclohexane yielded the 1β-hydroxy-6β-dihydrocinnamate **10** (1.3 g): bp 205° (bath) (0.04 Torr); nmr δ 0.85 (3, s, C-8a Me), 2.71 (4, A_2B_2 m, $C_6H_5CH_2CH_2CO$), 3.42 (1, m, C-1α H), 4.62 (1, m, C-6α H), and 7.20 ppm (5, s, C_6H_5).

Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.91. Found: C, 75.93; H, 8.75.

3α,6β-Dihydroxytropane (12).—6β-Hydroxytropinone (**11**, 1 g, 6.4 mmol, prepared according to the procedure of Nedenskov and Clauson-Kaas[11]) and Raney nickel W-4 (2 g) in ethanol (20 ml) were kept at 21° under hydrogen (50 psi) for 14 hr. The filtered solution was then evaporated and the product was recrystallized from ethyl acetate to give the 3α,6β-diol **12** (800 mg): mp 183–184° (lit.[12] mp 179–180°); nmr (acetone-d_6) δ 2.46 (3, s, NCH_3), 3.97 (1, t, J = 5 Hz, C-3β H), and 4.73 and 4.81 ppm (1, d of d, J = 4 Hz, C-6α H).

3α,6β-Bis(dihydrocinnamoyloxy)tropane (13).—The above diol **12** (600 mg, 3.8 mmol) and freshly distilled dihydrocinnamoyl chloride were heated for 6 hr at 100°. The cooled reaction mixture was then neutralized with aqueous sodium carbonate and extracted with ethyl acetate (3 × 50 ml). The dried ($MgSO_4$) extract was evaporated and the solid obtained was recrystallized from ethyl acetate–ethanol to give **13** as its hydrochloride salt (800 mg): mp 166–168°; nmr (acetone-d_6 + D_2O) δ 2.5–3.0 (8, m, $C_6H_5CH_2CH_2CO$), 3.03 (3, s, NCH_3), 5.17 (1, t, J = 5 Hz, C-3β H), 5.56 and 5.64 (1, d of d, J = 4 Hz, C-6α H), and 7.4 ppm (10, s, C_6H_5).

Anal. Calcd for $C_{26}H_{32}NO_4Cl$: C, 68.18; H, 7.04. Found: C, 68.14; H, 6.86.

3α-Dihydrocinnamoyloxy-6β-hydroxytropane (14).—The diester **13** (650 mg, 1.42 mmol), acetone (30 ml), and 0.1 N aqueous sodium hydroxide (100 ml) were stirred at 30° for 6 hr. The mixture was then neutralized with 1 N aqueous hydrochloric acid, concentrated, and saturated with sodium carbonate. Extraction with ethyl acetate (3 × 50 ml) yielded a solid (310 mg) which on recrystallization from ether–hexane gave 3α-dihydrocinnamoyloxy-6β-hydroxytropane (**14**, 250 mg): mp 94°; nmr δ 2.50 (3, s, NCH_3), 2.78 (4, A_2B_2 m, $C_6H_5CH_2CH_2CO$), 4.33 and 4.41 (1, d of d, J = 4 Hz, C-6α H), 5.0 (1, t, J = 5 Hz, C-3β H), and 7.30 ppm (5, s, C_6H_5).

Anal. Calcd for $C_{17}H_{23}NO_3$: C, 70.56; H, 8.01. Found: C, 70.72; H, 8.12.

6β-Acetoxy-3α-dihydrocinnamoyloxytropane (15). A. From 14.—The 3α-dihydrocinnamate-6β-ol **14** (150 mg, 0.52 mmol) in acetyl chloride (2 ml) was warmed at 35° for 3 hr. The mixture was then evaporated to dryness and recrystallized from ethyl acetate–ethanol or acetone–ethanol to give the hydrochloride salt of **15** (110 mg): mp 155–156°; nmr δ 2.10 (3, s, $COCH_3$), 2.82 (4, A_2B_2 m, $C_6H_5CH_2CH_2CO$), 3.00 (3, s, $^+NCH_3$), 5.16 (1, "t," C-3β H), 5.44 and 5.53 ppm (1, d of d, J = 4 Hz, C-6α H), and 7.30 ppm (5, s, C_6H_5).

Anal. Calcd for $C_{19}H_{26}O_4NCl$: C, 62.02; H, 7.13. Found: C, 62.09; H, 7.36.

The above hydrochloride (1 g) in water (3 ml) saturated with K_2CO_3 was continuously extracted with ether for 15 hr. Evaporation of the dried ($MgSO_4$) ether solution yielded 6β-acetoxy-3α-dihydrocinnamoyloxytropane (**15**, 0.9 g): nmr δ 2.02 (3, s, $COCH_3$), 2.46 (3, s, NCH_3), 2.87 (4, A_2B_2 m, $C_6H_5CH_2CH_2CO$), 3.17 (2, broad, C-1 and C-5 H), 5.01 (1, t, J = 5 Hz, C-3β H), 5.32 and 5.40 (1, d of d, J = 4 Hz, C-6α H), and 7.23 ppm (5, s, C_6H_5).

B. From 17.—A solution of 6β-acetoxy-3α-hydroxytropane (**17**, 3 g, 15 mmol) and dihydrocinnamoyl chloride (3 g, 18 mmol) in dry ether (30 ml) was stirred at 21° for 15 hr. The precipitated hydrochloride salt of **15** formed was filtered off and recrystallized from acetone–ethanol. The sample, mp 155–156°, obtained, which was identical in all respects with the previously characterized material, was converted into the free base **15** by the procedure outlined in A above.

6β-Acetoxytropinone (16).—A solution of 6β-hydroxytropinone[11] (**11**, 1.6 g, 10.3 mmol) in acetic anhydride (10 ml) was stirred at 20° for 14 hr. Water (10 ml) was then added and the mixture was evaporated to dryness. The residue was dissolved in water (3 ml) containing K_2CO_3 (1 g) and the solution was extracted continuously with ether for 15 hr. Evaporation of the dried ($MgSO_4$) ether extract gave 6β-acetoxytropinone (**16**, 2 g): bp 110° (0.03 Torr); nmr δ 2.05 (3, s, $COCH_3$), 2.65 (3, s, NCH_3), 3.57 (2, broad, C-1 and C-5 H), and 4.97 ppm (1, t, J = 5 Hz, C-6α H).

Anal. Calcd for $C_{10}H_{15}O_3N$: C, 60.88; H, 7.68. Found: C, 61.12; H, 7.72.

6β-Acetoxy-3α-hydroxytropane (17).—6β-Acetoxytropinone (**16**, 3 g, 15 mmol) in absolute ethanol (30 ml) was hydrogenated (50 psi) for 14 hr in the presence of W-4 Raney nickel (5 g). The catalyst was then removed and the solvent was evaporated to give 6β-acetoxy-3α-hydroxytropane (**17**, 2.9 g): bp 122° (0.3 Torr); nmr δ 2.03 (3, s, $COCH_3$), 2.47 (3, s, NCH_3), 2.86 (1, s, OH), 4.05 (1, t, J = 5 Hz, C-3β H), and 5.64 and 5.56 ppm (1, d of d, J = 4 Hz, C-6α H).

Anal. Calcd for $C_{10}H_{17}O_3N$: C, 60.26; H, 8.61. Found: C, 60.28; H, 8.67.

Hydroxide-Ion Hydrolysis of 1β-Acetoxy-6β-dihydrocinnamoyloxy-8aβ-methyl-1,2,3,4,4aα,5,6,7,8,8a-decahydronaphthalene (5).—To the diester **5** (16.7 mg, 0.047 mmol) in methanol (2 ml) was added 0.1 N aqueous sodium hydroxide (0.47 ml, 0.047 mmol) and the solution was stirred at 20° for 6 hr under nitrogen. The mixture was then diluted with water (10 ml) and thoroughly extracted with ethyl acetate. The product contained the starting diester **5** (1.6 mg), the 1β-acetate **3** (5.7 mg), and the 1β,6β-diol **18** (3 mg). Each compound was identified by comparison with authentic samples.

Selective Hydroxide-Ion Hydrolysis of the C-6β-Ester Functions of 13 and 15. A. Of 6β-Acetoxy-3α-dihydrocinnamoyloxytropane (15).—The acetate dihydrocinnamate **15** (100 mg, 0.27 mmol) in acetone (5 ml) and 0.1 N aqueous sodium hydroxide (20 ml) was stirred at 30° for 6 hr. After it was neutralized with 1 N hydrochloric acid, the mixture was concentrated, saturated with sodium carbonate, and extracted with ethyl acetate (2 × 25 ml). Evaporation of the dried ($MgSO_4$) ethyl acetate solution followed by recrystallization from ether–hexane gave the 6β-hydroxydihydrocinnamate **14** (60 mg), mp 94°, identical in all respects with the sample prepared previously.

B. Of 3α,6β-Bis(dihydrocinnamoyloxy)tropane (13).—This was carried out as described in the reaction sequence **13** → **14**.

α-Chymotrypsin-Catalyzed Hydrolyses. Preparative Scale Experiments.—The general procedure followed was as detailed previously.[5] The diester substrates were suspended in 0.1 M aqueous potassium chloride at 25° in a magnetically stirred Radiometer pH-Stat reaction vessel of up to 100-ml capacity fitted with a rubber stopper through which a combination electrode, needle of the sodium hydroxide filled syringe, and nitrogen line were introduced. The pH-Stat was set to maintain a pH of 7.8 automatically.[20] The desired hydrolyses were initiated by adding sufficient amounts of a stock solution of α-chymotrypsin in 0.1 M aqueous potassium chloride to give a concentration in the reaction mixture of 1 mg/ml. After the addition of ~1 equiv of base, the rate of hydrolysis slowed markedly for the better substrates. The reaction mixtures were often homogeneous by this time and were then extracted with a suitable organic solvent and the products were isolated and purified. Except in the case of the kojic acid esters,[21] no hydrolysis was detectable at pH 7.8 in the absence of the enzyme. For the more hydrophobic compounds, small amounts (2–5%) of acetonitrile sometimes increased the rate of hydrolysis. For reactions requiring >10 hr for completion, autolysis of the enzyme began to become significant and a correction for the additional base uptake involved was made. In general, however, the enzyme activity remained relatively constant during the course of selective dihydrocinnamate cleavage for the better substrates.

Kinetic Studies.—All studies were performed at 25° in 0.1 M aqueous potassium chloride solutions at pH 7.8. The relative rates of hydrolyses of the substrates under the heterogeneous preparative-scale conditions were determined from the initial velocities of reaction mixtures 0.7 mM in substrate and 3.8 × 10^{-6} M in α-chymotrypsin. The base used was 0.01 N aqueous sodium hydroxide. For the decalin esters, for which the addition of organic cosolvents was beneficial in some cases, 5% acetonitrile was added.

The kinetic constants for the 6β-hydroxy-1β-dihydrocinnamate **7**, the 1β-hydroxy-6β-dihydrocinnamate **10**, and the tropane-6β-acetate–3α-dihydrocinnamate **15** were determined by the usual pH-Stat procedure, Enzyme concentrations were determined by the spectrophotometric method of Cunningham.[35] For **7** and **10**, homogeneous solutions were obtained on addition of 20% acetonitrile as cosolvent. Concentrations in the 0.17–0.9 mM range

(35) L. W. Cunningham, *J. Biol. Chem.*, **207**, 443 (1954).

were studied using an enzyme concentration of 3.7×10^{-6} M. For comparison purposes, the k_{OH} values for both compounds were also determined in the same solvent composition using pH 11 solutions. The data for methyl dihydrocinnamate in 20% acetonitrile were obtained using a substrate concentration range of 1–7 mM and α-chymotrypsin at 4.15×10^{-7} M. For **15** no cosolvent was required; the enzyme concentration used was 0.8×10^{-6} M with a substrate range of 1–10 mM. The kinetic data were subjected to least-squares regression analysis and the k_{cat} and K_m constants were computed using the reciprocal method of Lineweaver and Burk.[36]

α-Chymotrypsin-Catalyzed Hydrolysis of 5.—The 1β-acetoxy-6β-dihydrocinnamoyloxydecalin **5** (140 mg, 0.39 mmol) was hydrolyzed at 25° using α-chymotrypsin (10 mg) in 0.1 M aqueous potassium chloride (10 ml) containing 2% acetonitrile by the general preparative procedure; 0.5 N aqueous sodium hydroxide was used to maintain the pH at 7.8. After 24 hr, when 0.3 mmol of base had been taken up, the rate slowed dramatically and the reaction mixture was extracted with ether (3 × 50 ml). Glc analysis showed that the extract contained 0.31 mmol of the 1β-acetate product of specific 6β cleavage and 0.08 mmol of the starting diester. Tlc separation of the mixture using ethyl acetate–benzene (1:6) as developing solvent yielded the 1β-acetoxy-6β-hydroxydecalin **3** (46 mg) which was identical with an authentic sample. None of the 6β-dihydrocinnamate **10** was detected. Extraction of the acidified aqueous mother liquors of the reaction with chloroform (3 × 50 ml) give dihydrocinnamic acid (37 mg, 0.25 mmol).

α-Chymotrypsin-Catalyzed Hydrolyses of 6.—The 1β-acetate-6α-dihydrocinnamate **6** (10 mg, 0.27 mmol) in 0.1 M aqueous potassium chloride (5 ml) was treated with α-chymotrypsin (5 mg) as described above. Aqueous sodium hydroxide (0.1 N) was the base used and after 24 hr, when ~2 equiv of alkali had been consumed, the reaction was worked up as before. (The excess base uptake is due to autolysis of the enzyme.) Glc analysis showed hydrolysis to have been 96% selective for the 6α position, and the 1β-acetoxy-6α-hydroxy compound **4** (3 mg), identical with authentic material, was obtained following tlc purification.

α-Chymotrypsin-Catalyzed Hydrolysis of 15.—The hydrochloride salt of 6β-acetoxy-3α-dihydrocinnamoyloxytropane (**15** HCl, 1.55 g, 4.22 mmol) in 0.1 M aqueous potassium chloride (60 ml) was placed in the pH-Stat under the standard conditions. The pH of the reaction solution (~5.5) was adjusted to 7.8 with 1 N aqueous sodium hydroxide (2.3 mmol required), and α-chymotrypsin (60 mg) was then added. The hydrolysis stopped after 4 hr (0.2 mmol of base uptake). The reaction mixture was saturated with potassium chloride and extracted continuously with ether for 24 hr. Evaporation of the dried ($MgSO_4$) ether extract yielded the dihydrocinnamate salt of 6β-acetoxy-3α-hydroxytropane (100 mg). The aqueous solution was then saturated with potassium carbonate and again continuously extracted with ether for 24 hr. Evaporation of the dried ether solution gave 6β-acetoxy-3α-hydroxytropane (**17**, 780 mg, 3.9 mmol), identical in all respects with an authentic sample. A further 62 mg (0.21 mmol) of **17** was obtained by treatment of the dihydrocinnamate salt with aqueous potassium carbonate.

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Registry No.—**1** 1β-alcohol derivative, 878-47-7; **2**, 41163-95-5; **2** 1β-OAc derivative, 36925-35-6; **2** 1β-ODHC derivative, 41163-97-7; **2** 1β-OTHP derivative, 38538-76-0; **3**, 41163-98-8; **4**, 41163-99-9; **5**, 41164-00-5; **6**, 41164-01-6; **7**, 41164-02-7; **8**, 41164-03-8; **10**, 41164-04-9; **10** 1β-OTHP derivative, 41172-09-2; **11**, 5932-53-6; **12**, 41164-06-1; **13**, 41164-07-2; **13** HCl, 41164-08-3; **14**, 41164-09-4; **15**, 41164-10-7; **15** HCl, 41164-11-8; **16**, 41164-12-9; **17**, 41164-13-0; dihydrocinnamoyl chloride, 645-45-4.

(36) H. Lineweaver and D. Burk, *J. Amer. Chem. Soc.*, **56**, 658 (1934).

The Bimolecular Decarboxylative Self-Condensation of Oxaloacetic Acid to Citroylformic Acid and Its Conversion by Oxidative Decarboxylation to Citric Acid

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Oxaloacetic acid undergoes a bimolecular decarboxylative self-condensation to give 4-carboxy-4-hydroxy-2-ketohexane-1,6-dioic acid (**1**) (citroylformic acid). The reaction goes in high yield at pH 3–7, at 25–30°, and in aqueous media. The acid is converted to citric acid by oxidative decarboxylation and to its lactone by dehydration but is not obtainable from the previously described oxalocitrolactone ester structures.

Although the chemistry of oxaloacetic acid has been studied in considerable detail, there are unexplained observations in some of the reported data. These are reported in studies of the nmr[1] and uv[2] spectra in which anomalous behavior is attributed to unidentified dimeric structures. Since dimerization has been noted for pyruvic acid, and characterized in some detail,[3–7] it seems reasonable to postulate the dimerization[8] of oxaloacetic acid. Furthermore, because such dimeric structures are formed under physiological conditions (aqueous, pH 7) and are, therefore, possibly significant in biological systems, and since the extensive studies of the kinetics and mechanism of the decarboxylation of oxaloacetic acid[9] (based on the rate of evolution of carbon dioxide) should be interpreted for the possibility of rate-determining dimerization preceding decarboxylation,[10] additional information on this dimerization reaction is desirable. We wish to describe the results of a study of the decarboxylative self-condensation of oxaloacetic acid to citroylformic acid (**1**) (CFA) and isolation and characterization of this new acid.

Oxaloacetic acid undergoes a bimolecular aldol-type condensation in aqueous solution at room temperature (25–30°) and pH 3–7 with the loss of carbon dioxide to

(1) W. D. Kumler, E. Kun, and J. N. Shoolery, *J. Org. Chem.*, **27**, 1165 (1962).

(2) C. R. Goucher and E. H. Strikland, *U. S. Army Med. Res. Lab. Rep.*, Dept. No. 571 (1964); *Chem. Abstr.*, **62**, 14489 (1965).

(3) L. Wolff, *Justus Liebigs Ann. Chem.*, **305**, 154 (1899).

(4) A. W. K. Dedong, *Recl. Trav. Chim. Pays-Bas*, **20**, 81 (1901).

(5) E. Waldman, V. Prey, and F. Jellinek, *Monatsh. Chem.*, **85**, 872 (1953).

(6) V. Prey, E. Waldman, and H. Besbalk, *Monatsh. Chem.*, **86**, 408 (1955).

(7) D. L. Leussing and C. K. Stanfield, *J. Amer. Chem. Soc.*, **86**, 2805 (1964).

(8) F. L. Breusch and R. Tulus, *Rev. Fac. Sci. Univ. Istanbul*, **6**, 144 (1941); *Chem. Abstr.*, **37**, 4366 (1943).

(9) E. Gelles, *et al.*, *J. Chem. Soc.*, 4136 (1956); 3673, 3683, 3689 (1958); and references cited therein.

(10) This is especially true since many of the decarboxylation studies are designed to evaluate the effect of metal ions which are known to catalyze aldol dimerization of α-keto acids. See E. H. Abbott and A. E. Martell, *J. Amer. Chem. Soc.*, **91**, 6931 (1969).