Anal. Calcd for $C_{29}H_{50}O_2S$: C, 75.28; H, 10.89; S, 6.92. Found: C, 74.88; H, 10.95; S, 7.16.

Equilibration of 5β-Cholestan-4-one Using Methanolic Potassium Hydroxide.—A solution of 5β-cholestan-4-one (66 mg) in 10% methanolic potassium hydroxide solution was heated to reflux for 18 hr. The crude product was isolated by extraction with ether (3 times), and evaporation of the ethereal extract after washing with water and drying (Na_2SO_4). Preparative tlc (petroleum ether–ethyl acetate, 9:1) gave pure 5α-cholestan-4-one (53 mg) and pure 5β-cholestan-4-one (11 mg), by elution of the scraped out zones with ethyl acetate. The products were identified by tlc, infrared comparison, and melting point, and mixture melting point determination.

Equilibration of Cholestan-4-one 3-Ethylene Monothioketals 8b and 8d with Potassium Hydroxide in Methanol. A.—The 5α-cholestan-4-one derivative **8b** (10 mg) was dissolved in 10% methanolic potassium hydroxide solution (5 ml) and the solution was heated under reflux for 4 hr. Monitoring of the reaction solution by tlc (petroleum ether–ethyl acetate, 19:1) showed no change, and the reaction mixture was worked up by dilution with water, extraction with ether, and evaporation of the dried (Na_2SO_4) ethereal extract. The crude residue (9.3 mg) was unchanged **8b** as shown by tlc, infrared comparison, and melting point, and mixture melting point determination.

B.—The 5β-cholestan-4-one derivative **8d** (6 mg) was dissolved in 10% methanolic potassium hydroxide solution (4 ml) and the solution was heated under reflux. Monitoring of the reaction solution by tlc (petroleum ether–ethyl acetate, 19:1) showed that no **8d** was present after 2 hr, but that a new compound was present with an R_F identical with that of the 5α compound **8b**. After 3 hr the situation was unchanged, and work-up of the reaction mixture as for A above gave crude product (5.5 mg) which proved identical with compound **8b** as shown by tlc, infrared comparison, and melting point, and mixture melting point determination.

Equilibration of Cholestan-4-one 3-Ethylene Monothioketals 8a and 8c with Potassium Hydroxide in Methanol. A.—A solution of the 3-monothioketal **8a** (9 mg) in 10% methanolic potassium hydroxide solution (5 ml) was heated under reflux for 2.5 hr. Monitoring of the reaction solution by tlc (petroleum ether–ethyl acetate, 19:1) had shown that no further change occurred after 2-hr reflux. Work-up as for the previous equilibration and preparative tlc of the crude product (8 mg) gave pure starting material **8a** (1.0 mg) and pure compound **8c** (6.0 mg), identified in the former case by tlc, melting point, and mixture melting point determination, and in the latter case by the above criteria and also by infrared comparison.

B.—A solution of the 3-monothioketal (**8c**, 100 mg) in 10% methanolic potassium hydroxide solution (50 ml) was heated under reflux for 2.5 hr. Work-up as for the previous equilibrations gave crude product (95 mg) which was separated by preparative tlc into pure starting material **8c** (78 mg) and pure compound **8a** (12 mg). Identification in each case was by tlc, infrared comparison, melting point, and mixture melting point determination.

Registry No.—**2a**, 18897-72-8; **2b**, 18897-73-9; **3a**, 28876-03-1; **3b**, 28876-04-2; **3c**, 28876-05-3; **7**, 28876-06-4; **8a**, 18897-78-4; **8b**, 17021-85-1; **8c**, 18897-79-5; **8d**, 18897-77-3; **9**, 28856-58-8; **11a**, 18897-74-0; **11b**, 18897-75-1; **12**, 28856-61-3; **13**, 28856-62-4; **14a**, 18897-83-1; **14b**, 18897-82-0; **15**, 18897-80-8; **16**, 18897-81-9; 5α-cholestan-4β-ol, 566-50-7; cholestane-3,4-dione bis(ethylene monothioketal), 28856-67-9.

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β-Carbonylamides in Peptide Chemistry. Synthesis of Optically Active Peptides from *N*-Acetoacetylamino Acids *via* 2-Acetonylidenoxazolidin-5-ones[1]

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N-Acetoacetylamino acids react with dicyclohexylcarbodiimide yielding 2-acetonylidenoxazolidin-5-ones. These condense in turn with nucleophiles producing amides and peptides with retention of configuration.

In contrast to the widespread tendency of activated *N*-acylamino acids to racemize under conditions suitable for peptide synthesis,[2] we recently found that *N*-acetoacetylamino acids (AcA-aa) (**1**) yield optically pure peptide derivatives under certain conditions;[3] furthermore, the acetoacetyl protecting group can be selectively cleaved with hydroxylamine under very mild conditions.[3,4]

To explore the reasons for this retention of configuration, we examined the behavior of some AcA-aa when treated with dicyclohexylcarbodiimide (DCCI) and isolated reactive acylating agents that we regard as 2-acetonylidenoxazolidin-5-ones. Their optical stability and tendency to condense with nucleophiles have been compared with similar properties of some related azlactones (**2**).

Representative *N*-AcA-aa (**1**) were reacted with DCCI under the conditions used in peptide synthesis but omitting a nucleophilic partner. A molar amount of dicyclohexylurea (DCU) was formed, while the optical activities of the solutions shifted to higher positive values. Prompt lyophylization of the solutions yielded solid, frequently crystalline products.

The uv spectra exhibited a strongly conjugated chromophore [$\lambda_{max}^{dioxane}$ near 285 nm (ε *ca.* 10,000)] ruling out the formation of anhydrides;[5] in the ir spectra, a strong absorption at 1835–1840 cm^{-1} accounted for the presence of a carbonyl group in a strained lactone ring. Finally, the nmr spectra showed absorptions that could more satisfactorily be ascribed to 2-acetonylidenoxazolidin-5-ones (**3**) than to 2-acetonyl-2-oxazolin-5-ones (**2′** and possible tautomers **2″**, **2‴**), as might be expected since β-aminoenones are more stable than the isomeric β-imino ketones.[6] Furthermore, evidence has been obtained that β-aminoenones

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(2) G. T. Young, "Peptides 1967," H. C. Beyerman, *et al.*, Ed., North-Holland, Amsterdam, 1967, p 55.

(3) C. Di Bello, F. Filira, V. Giormani, and F. D'Angeli, *J. Chem. Soc. C*, 350 (1969), and references cited therein.

(4) A. Marzotto, P. Pajetta, L. Galzigna, and E. Scoffone, *Biochim. Biophys. Acta*, **154**, 450 (1968).

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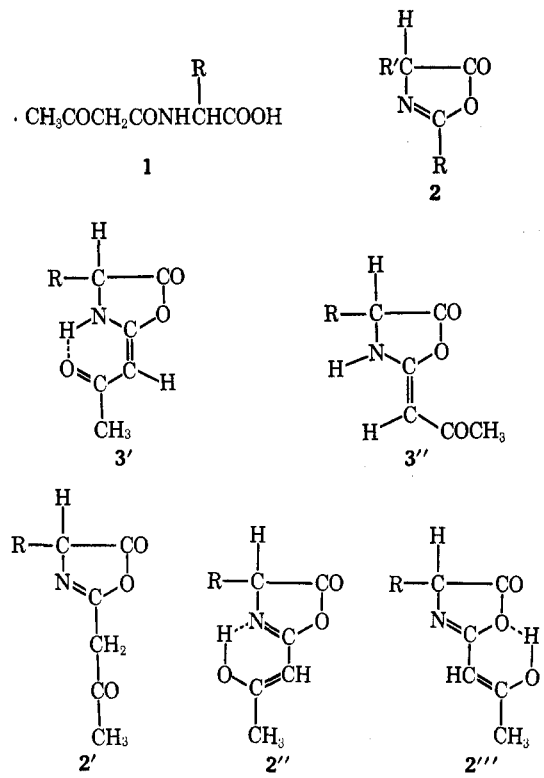
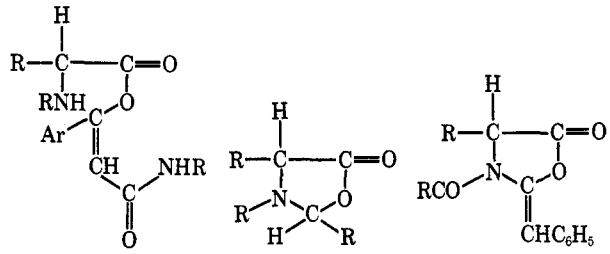
are formed also from *N*-methyl AcA-aa.[7a] However, the nmr spectra of the present compounds display signals of only one vinyl proton, instead of two as required if both stereoisomers **3′** and **3″** were present.[7b] The single isomer suggested by this feature should be **3′** which can be stabilized by intramolecular hydrogen bonding.



The 2-acetonylidenoxazolidin-5-ones **3a–d** were optically stable for at least a few days when stored dry at room temperature in the solid state after lyophylization. In dioxane solution at 20°, the loss of optical activity was faster and some influence of the substituent at C_4 was observed; no conclusions regarding relative optical stabilities can be drawn at present. When the optically active 2-acetonylidenoxazolidin-5-ones were reacted with nucleophiles, optically pure condensation products were obtained, as demonstrated by independent synthesis or glpc analysis.

Qualitative rate experiments showed that a 2-acetonylidenoxazolidin-5-one (**3**) condenses with aniline, valine methyl ester, or benzylamine somewhat faster than an azlactone (**2**). It is known, on the other hand, that azlactones racemize at a faster rate than they condense with nucleophiles.[8]

The similarity of compounds **3a–d** to Woodward's enol esters **4**[9] as well as to N-substituted oxazolidin-5-ones **5** and *N*-acyl-2-benzylidenoxazolidin-5-ones **6** should be pointed out.[10] All have acylating properties, and compounds of type **4** and **5** were shown to yield optically active peptides and amides.[9,11]



Whether 2-alkylidenoxazolidin-5-ones (**3**) indeed play a role as intermediates in the condensation of AcA-aa with nucleophiles by means of DCCI has not yet been established. The observed retention of configuration may be due to quenching of the path involving oxazolin-5-ones (**2**), whose intermediacy may seriously affect the optical purity in the condensation step. Further studies on structural features and possible applications of 2-acetonylidenoxazolidin-5-ones (**3**) are in progress.

Experimental Section[12,13]

Acetoacetylamino Acids.—The following acids were used: AcA-L-Ala-OH, mp 92–93°, $[\alpha]$ −4.6°, λ_{max} 245 nm (ϵ 3830); AcA-L-Val-OH, mp 124–125°, $[\alpha]$ +7.4°, λ_{max} 245 nm (ϵ 2060); AcA-L-Leu-OH, mp 124–125°, $[\alpha]$ −16.3°; λ_{max} 245 nm (ϵ 2100);[3] AcA-L-Phe-OH, mp 109–110°, $[\alpha]$ +68.2°, λ_{max} 245 nm (ϵ 2340), prisms from ethyl acetate–petroleum ether (bp 30–60°). *Anal.* Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.6. Found: C, 63.35; H, 6.06; N, 5.8.

Optically Active Azlactones (2). 2-Phenyl-4-isobutyl-2-oxazolin-5-one (2a).—A solution of *N*-benzoyl-L-leucine[14a] (1.125 g, 0.005 mol) in 10 ml of anhydrous dioxane, mixed with DCCI (1.03 g, 0.005 mol) and allowed to stand 2 hr, gave 98% DCU. A sample of the solution, diluted with dioxane to a 2% concentration, gave $[\alpha]$ −63.8°. Lyophilization yielded colorless prisms (0.85 g, 75%): mp 51–52°; ir 1830 (s, CO), 1660 (s, C=N), 1580 cm^{-1} (w). *Anal.* Calcd for $C_{13}H_{15}NO_2$: C, 71.86; H, 6.96; N, 6.45. Found: C, 71.17; H, 7.25; N, 6.56.

The racemic product (mp 56–57°) had been previously obtained from *N*-benzoyl-DL-leucine.[14b,15]

2-Phenyl-4-isopropyl-2-oxazolin-5-one (2b) was obtained from *N*-benzoyl-L-valine[16a] in the same manner as the above compound: ir 1830 cm^{-1} (s, CO); $[\alpha]$ −77.8°.[16b]

2-Acetonyliden-4-methyloxazolidin-5-one (3a) and Analogous Products (3b–d). A.—A sample of AcA-L-alanine (0.45 g, 0.0026 mol) was dissolved in 5 ml of anhydrous dioxane and mixed with DCCI (0.53 g, 0.0026 mol). After 2 hr at room temperature, precipitation of DCU was complete; it was filtered off; and the solution was chilled, lyophilized, and dried over P_2O_5 (0.394 g, 98%). The product was a colorless microcrystalline solid: mp 113–116°; $[\alpha]$ +49.5°; λ_{max} 284 nm (ϵ 13,000); ir 3280

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(12) Optical activities were measured with a Perkin-Elmer 141 polarimeter; maximum values observed are reported as $[\alpha]^{25}D$ for 2% solutions in dioxane, if not otherwise stated. Melting points were taken in a Kofler apparatus. Spectra were measured as follows: ir, Perkin-Elmer Model 337 double beam recording spectrophotometer equipped with sodium chloride optics (in CCl_4); uv, Coleman Hitachi 124 double beam recording spectrophotometer (in dioxane); nmr, Perkin-Elmer R12 spectrometer (in $CDCl_3$). For thin layer chromatography (tlc), precoated plates of silica gel Merck F 254 were used, with ethyl acetate–benzene (2:1) as eluent. We acknowledge the skillful technical assistance of Mr. Adriano Mencini.

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(NH), 1840 (s, ring CO), 1720 (w), 1670 (s, conjd CO), 1620 (w), 1470 cm^{-1}; nmr δ 8.8 (NH), 5.2 (=CH), 4.3 (C_4H), 2.1 ($COCH_3$) 1.5 (d, C_4 CH_3). *Anal.* Calcd for $C_7H_9NO_3$: C, 54.19; H, 5.85; N, 9.07. Found: C, 54.52; H, 6.33; N, 9.33.

Compounds **3b–d**, prepared from AcA-L-valine, -L-leucine, and -L-phenylalanine, had analogous properties, minor differences being those expected for the group at C_4. The samples, dried to constant weight and not recrystallized, were colorless microcrystalline solids that could be stored for days in a drybox with no change; yields were almost quantitative. The nmr spectra indicated minor contamination by the parent AcA-aa.

3b [R = $CH(CH_3)_2$]: mp 80–85°; [α] +70°; λ_{max} 283 nm (ϵ 11,500). *Anal.* Calcd for $C_9H_{13}NO_3$: C, 59.01; H, 7.15; N, 7.64. Found: C, 58.99; H, 7.13; N, 7.71.

3c [R = $CH_2CH(CH_3)_2$]: mp 107–109°; [α] +63.5°; λ_{max} 284 nm (ϵ 11,000). *Anal.* Calcd for $C_{10}H_{15}NO_3$: C, 60.89; H, 7.67; N, 7.10. Found: C, 60.87; H, 7.56; N, 7.35.

3d (R = $CH_2C_6H_5$): mp 130–133°; [α] +92°; λ_{max} 285 nm (ϵ 12,500). *Anal.* Calcd for $C_{13}H_{13}NO_3$: C, 67.57; H, 5.67; N, 6.06. Found: C, 67.43; H, 5.50; N, 6.01.

B.—Similar reactions were carried out in anhydrous tetrahydrofuran, dichloromethane, ether, or dioxane. After filtration of the DCU, the solutions were promptly diluted to standard volumes. Uv and ir spectra and specific rotations were in most cases identical with those of the above crystalline products.

The nmr spectrum of a reaction mixture obtained from AcA-L-Val-OH in $CDCl_3$, after only 45 min incubation with 2 mol of DCCI and filtration of DCU, showed again a single vinyl peak (δ 5.1).

Samples of 2% dioxane solutions were stored at room temperature and the decrease of optical activity with time was followed. Whereas in some cases (compounds **3a** and **3b**) only ~20% optical activity was lost in 10 days, in others (compounds **3c** and **3d**) more than 50% was lost within 2 days, as was the case for the two azlactones (**2a,b**) used as references.

***N*-Acetoacetyl-L-leucylglycine Ethyl Ester.**—A sample of AcA-L-leucine (1.075 g, 0.005 mol) in 10 ml of anhydrous dioxane was added with DCCI (1.03 g, 0.005 mol). After 2 hr the DCU was filtered and rapidly washed with a little dioxane; the solution and washings were mixed under stirring with a solution of free glycine ethyl ester[17] (0.515 g, 0.005 mol) in 5 ml of dioxane and allowed to stand overnight. Upon lyophilization and trituration with petroleum ether, a colorless solid was obtained (1.39 g, 92%). It was freed from contaminating DCU and AcA-leucine by column chromatography (SiO_2, ethyl acetate–benzene 2:1), yielding the pure title compound (1.26 g, 84%), mp 87–88°, [α] −47.5° (2%, ethanol). This sample was identical with the one obtained upon acetoacetylation of H-L-Leu-Gly-OEt, obtained in turn *via* Z.[3]

***N*-Acetoacetyl-L-valyl-L-valine Methyl Ester.**—A solution containing about 5 mmol of 2-acetonyliden-4-isopropyloxazolidin-5-one (**3b**) in 10 ml of dioxane, obtained from 0.005 mol each of AcA-L-valine and DCCI and freed from DCU, was treated with H-L-Val-OMe (5 mmol) in 15 ml of dioxane and left overnight. By working up the mixture as above, AcA-L-Val-L-valine was obtained (98%): mp 55–58°; [α] −53.2° (2%, ethanol); tlc R_f 0.34 ($FeCl_3$). A sample was deacetoacetylated and then trifluoroacetylated, yielding N-TFA-L-Val-L-Val-OMe.[3] Glpc[18a] showed contamination by no more than 1% of the DL isomer.

Acetoacetyl-L-leucine-*N*-benzylamide.—A solution containing about 10 mmol of 2-acetonyliden-4-isobutyloxazolidin-5-one (**3c**) in 20 ml of dioxane was obtained as described above from 10 mmol each of AcA-L-leucine and DCCI. After removal of DCU, the solution was treated with benzylamine (1.7 g, 0.01 mol), concentrated, and triturated with petroleum ether (2.7 g, 90%): colorless crystals; mp 112–113°; [α] −43.5° (2%, ethanol); R_f 0.5 (I_2NaN_3). *Anal.* Calcd for $C_{15}H_{24}N_2O_3$: C, 67.08; H, 7.95; N, 9.20. Found: C, 66.61; H, 8.15; N, 8.87.

A sample of the amide was deacetoacetylated with hydroxylamine[3] and reacted with benzyloxycarbonyl chloride. *Z*-Leucine benzylamide was obtained, identical with an authentic sample, mp 112–113°, [α] −17.2° (1.2%, ethanol).[18b]

Comparative Rate Experiments. Condensation of 2-Acetonylidenoxazolidin-5-ones and Oxazolin-5-one with Nucleophiles. A. Condensation with Aniline.—In a calibrated flask, a sample of 2-acetonyliden-4-isobutyloxazolidin-5-one (**3c**) (37.5 mg, 0.188 mmol) in 2–3 ml of dioxane was added with aniline (876 mg, 9.4 mmol) under stirring, and the volume was brought up to 5 ml (final concentrations, 0.0376 and 1.88 *M*, respectively). The drop of the concentration of **3c** was followed by reading this solution at 1840 cm^{-1}, using as a reference a solution of aniline of the same concentration (0.2-mm KBr cells). A straight line was obtained by plotting the logarithms of absorbances *vs.* time up to 1660 sec. The slope gave $K_{obsd} = 2.9 \times 10^{-4}$ corresponding to $t_{1/2}$ of 2375 sec.

A dioxane solution of 2-phenyl-4-isobutyl-2-oxazolin-5-one (**2a**) (41 mg, 0.188 mmol) and aniline, having the same concentration of the above experiment, was analyzed as described above at 1834 cm^{-1}. A straight line was obtained up to 5000 sec. $K_{obsd} = 0.898 \times 10^{-4}$ sec^{-1}; $t_{1/2}$, 7736 sec.

B. Condensation with Valine Methyl Ester.—A similar experiment was carried out with the above compounds (**3c** and **2a**) using free valine methyl ester as nucleophile (solution 0.204 *M* in **3c** or **2a** and 2.04 *M* in valine methyl ester); **3c** reacted within the time of mixing of the reagents ($t_{1/2}$ less than 10–15 sec), while **2a** condensed more slowly ($t_{1/2}$ 268 sec) (0.2-mm KBr cells).

C. Condensation with Benzylamine.—When **3c** and **2a** were reacted in the above conditions using benzylamine as nucleophile (solutions 0.109 *M* in **3c** or **2a** and benzylamine), **3c** gave $t_{1/2}$ of 10–15 sec, whereas **2a** gave $t_{1/2}$ of 454 sec (0.1-mm KBr cells).

Registry No.—**2a**, 28897-80-5; **2b**, 28897-81-6; **3a**, 28897-82-7; **3b**, 28897-83-8; **3c**, 28897-87-2; **3d**, 28897-88-3; AcA-L-Ala-OH, 3103-37-5; AcA-L-Val-OH, 3103-33-1; AcA-L-Leu-OH, 1803-64-1; AcA-L-Phe-OH, 17667-55-9; *N*-acetoacetyl-L-leucylglycine ethyl ester, 1803-65-2; *N*-acetoacetyl-L-valyl-L-valine methyl ester, 21761-28-4; *N*-acetoacetyl-L-leucine benzylamide, 28897-86-1.

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