In order to determine the optimum conditions for the formation of the diester, the effect of ratio of reactants and order of addition of the reagents on the yield of the diester was investigated. The slow addition of 1 mole of I to 2 moles of phosphoryl chloride gave no III, but to 1.2–1.4 moles of phosphoryl chloride gave III in 20–22% yield. Addition of 1 mole of phosphoryl chloride to 0.5–2 moles of I gave an improved yield of III (30–70%). The presence of a trace amount of moisture in the reaction mixture promoted the formation of III and lowered markedly the yield of II.

Experimental

Paper chromatography was undertaken with two solvent systems: solvent A, *n*-propyl alcohol–aqueous ammonia–water (20:12:3); solvent B, isopropyl alcohol–saturated aqueous ammonium sulfate–water (2:79:19).

Preparation of Diinosine Phosphate (III) from 2′,3′-O-Isopropylideneinosine (I) and Phosphoryl Chloride.—To a solution of I (100 g.) in dried pyridine (1.7 l.) cooled to −10° was added a solution of phosphoryl chloride (15 ml.) in dried pyridine (200 ml.) previously cooled to −10° with stirring in 7 min. After being kept for 1 hr. at −5°, the reaction mixture was poured into ice and water with vigorous stirring. After the pH of the solution was adjusted to 9 with aqueous sodium hydroxide, pyridine was removed under reduced pressure. Then the residual solution was acidified to pH 1.5 with hydrochloric acid and the acidic solution was warmed for about 1 hr. at 70° to remove the isopropylidene group. The resultant mixture was analyzed by paper chromatography. The per cent yields of II and III were estimated to be 30 and 70%, respectively, by the optical density at 250 mμ. The R_f values of II and III were 0.11 and 0.12 (solvent A) and 0.55 and 0.28 (solvent B), respectively. After neutralization with sodium hydroxide, the solution was concentrated to 500 ml., and most of II was separated by addition of an equal volume of ethyl alcohol. The filtrate was evaporated to 300 ml. and addition of 2 vol. of ethyl alcohol to the residue gave the precipitate which contained a large amount of III. The precipitate was dissolved in water, sodium ions were removed with IRC-50 (H^+ form) resin, and the solution was treated with barium hydroxide. The barium salt was recrystallized from water–ethyl alcohol repeatedly: $\lambda_{max}^{0.1\,N\,HCl}$ 250.4 mμ (ϵ_{250} 21,000), $\lambda_{max}^{0.1\,N\,NaOH}$ 255.0 mμ (ϵ_{250} 25,600), pK_a 4.01.

Anal. Calcd. for $C_{20}H_{22}O_{12}N_8P\cdot 0.5Ba$: C, 35.23; H, 3.51; N, 16.39; P, 4.1. Found: C, 35.85; H, 4.09; N, 16.34; P, 4.4.

Synthesis of Diinosine Phosphate from 2′,3′-O-Isopropylideneinosine (I) and *p*-Nitrophenyl Phosphorodichloridate (IV).—To a solution of I (2 g.) in pyridine (50 ml.), IV (1 g.) was added, and the mixture was kept overnight at room temperature. After the reaction mixture was poured into water (100 ml.), the pH of the solution was adjusted to 8.0, and pyridine was removed under reduced pressure with repeated addition of water. The concentrated solution (about 50 ml.) was acidified to pH 1.5 with 1 *N* hydrochloric acid and held for 1.5 hr. at 70° to remove the isopropylidene group. After the acidic solution was neutralized with sodium hydroxide, an additional 250 mg. of sodium hydroxide was added and the alkaline solution was heated for 2 hr. at 100° to remove the *p*-nitrophenyl group. The brownish solution was again slightly acidified with dilute hydrochloric acid and the liberated *p*-nitrophenol was extracted three times with ethyl acetate (50 ml.). Inosine, II, and III were detected by paper chromatography in the aqueous solution. After neutralization and concentration of the aqueous solution to 50 ml., 100 ml. of ethyl alcohol was added to precipitate II and III as sodium salts. I was purified as the barium salt, yield 1.35 g. (31%). The product was identical with the sample which was obtained by phosphorylation of I with phosphoryl chloride, by paper chromatography, paper electrophoresis, and infrared spectra.

5′-Tritylinosine (V).—Trityl chloride (6 g.) and anhydrous pyridine (50 ml.) were added to a solution of anhydrous inosine (5 g.) in dimethylformamide (100 ml.), and the mixture was heated for 2 hr. at 40°. After cooling to 0° the reaction mixture was poured into 500 ml. of ice and water with vigorous stirring. The precipitate was filtered, washed with benzene to remove trityl alcohol, and dried; yield 1.45 g. (crude 15.2%). Recrystallization from ethyl alcohol gave the pure crystal which melted at 207–210°. R_f value of V on paper chromatogram was 0.83 (solvent A).

Anal. Calcd. for $C_{27}H_{26}N_4O_5$: C, 68.22; H, 5.13; N, 10.97. Found: C, 67.91; H, 5.44; N, 10.98.

5′-Trityl-2′,3′-di-O-acetylinosine (VI).—Trityl chloride (6 g.) and anhydrous pyridine (50 ml.) were added to a solution of anhydrous inosine (5 g.) in dimethylformamide (100 ml.), and the mixture was heated for 2 hr. at 50°. To the resultant pale yellow solution was added acetic anhydride (15 ml.); then the mixture was kept for 18 hr. at room temperature. After the reaction mixture was poured into 500 ml. of ice and water, the deposited precipitate was filtered and washed with benzene to remove trityl alcohol; yield 3.7 g. (33%). Recrystallization from ethyl alcohol gave the pure crystal, m.p. 174°.

Anal. Calcd. for $C_{33}H_{30}N_4O_7$: C, 66.65; H, 5.09; N, 9.42. Found: C, 66.81; H, 5.26; N, 9.32.

The infrared spectrum of VI showed absorption band at 1240 cm.$^{-1}$ due to acetyl group.

2′,3′-Di-O-acetylinosine (VII).—A solution of VI (5 g.) in 80% acetic acid (100 ml.) was boiled for 30 min. After cooling, the precipitate of trityl alcohol was filtered off. The filtrate was concentrated to dryness, and the residue was extracted with chloroform. The residual sirup obtained by concentration of the solution of chloroform was crystallized from ethyl alcohol–petroleum ether; yield 0.6 g. (23%). Recrystallization from ethyl alcohol–petroleum ether gave pure crystal which melted at 215°.

Anal. Calcd. for $C_{14}H_{16}N_4O_7$: N, 15.92. Found: N, 15.75.

Reaction of 2′,3′-Di-O-acetylinosine (VII) with 2′,3′-Di-O-acetylinosine 5′-Phosphate Pyridinium Salt (VIII).—The pyridinium salt of II, which was prepared from the sodium salt of II (1 g.) by use of IR-120 (pyridinium form),[5] was dissolved in 40 ml. of dried pyridine and 5 ml. of acetic anhydride. The homogeneous solution was allowed to stand overnight and evaporated to dryness under reduced pressure in the presence of pyridine. To the residue 10 ml. of 90% pyridine in water was added to hydrolyze excess acetic anhydride. The pyridinium acetate produced was removed by lyophilization. The product was dissolved in pyridine and evaporated to dryness, and this procedure was repeated to remove traces of water. The resultant substance VIII, VI (1 g.), and dicyclohexylcarbodiimide (6 g.) were dissolved in 50 ml. of pyridine, and the solution was kept overnight with shaking. After addition of 100 ml. of water, precipitated dicyclohexylurea was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The residue was suspended in ethyl alcohol and a piece of metallic sodium was added to remove acetyl groups. In this solution II, III, inosine, and a trace of unknown spot (R_f 0.40 in solvent B) were found by paper chromatography (III could not be isolated in a pure state from the mixture).

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Enolic Concentrations in β-Keto Esters. Correlation of Bromometric and Ultraviolet Absorption Data[1]

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In an earlier paper,[3] we reported ultraviolet spectral data for a series of cyclic β-keto esters and appro-

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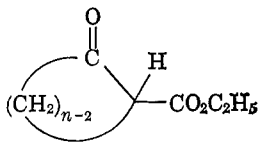
(2) American Chemical Society Scholar, 1962–1963.

(3) S. J. Rhoads, J. C. Gilbert, A. W. Decora, T. R. Garland, R. J. Spangler, and M. J. Urbigkit, *Tetrahedron*, **19**, 1625 (1963).

priate open-chain analogs. At that time, the enolic concentrations of the enolizable members of the series, estimated from the apparent molar absorptivities and an assumed molar absorptivity of *ca.* 12,000 for a *cis*-chelated enol, were shown to agree reasonably well with values reported by other workers[4-6] for the same or closely similar compounds and obtained by chemical methods. However, certain rather large discrepancies which did appear in some of the data suggested that a direct comparison of the chemical and spectral methods under a set of standardized conditions was advisable. In this paper we present the results of such a study for the series of 2-carbethoxycyclanones of ring members 5 through 12 (I-*n*). Included also are new values for the enol contents of the α-substituted ethyl acetoacetates II and III,[7] together with corresponding measurements for the isomeric enol ethers IV and V.



I-*n*, *n* = 5, 6, 7...12

$CH_3COCHRCO_2C_2H_5$
II, R = C_2H_5
III, R = sec-C_4H_9

$CH_3C(OR)=CHCO_2C_2H_5$
IV, R = C_2H_5
V, R = sec-C_4H_9

The enol contents of the compounds shown in Table I were, for the most part, determined by the "modified" bromometric method developed by Dieckmann for α-substituted β-keto esters.[4,9] In all cases, the measurements refer to 0.1 *M* solutions in absolute ethanol or cyclohexane which had been equilibrated in the dark at room temperature for at least 1 week before measurement. The spectral determinations were made on these same equilibrated solutions immediately following dilution to the proper concentrations for ultraviolet work. In this way, differences in enol content arising from further dilution were minimized. From the bromometric values and the apparent absorptivities (ϵ'), the true absorptivities (ϵ) were calculated. These are given in the last column of Table I.

It is gratifying that the absorptivities so derived do show a fairly constant order of magnitude (11,000–13,000) and one which is consistent with expectations for *cis*-chelated enolic systems. Eistert and Reiss[11] reported extrapolated estimates of 11,000 to 12,500 for the absorptivity of the enol of acetylacetone while Hammond, Borduin, and Guter,[12] in their study of diacylmethanes, reported an extrapolated value of 11,000 for enolic acetylacetone and measured values of 11,100 for diisobutyrylmethane enol and 12,500 for dipivaloylmethane enol. More recently, Korte and Wüsten[13] have presented bromometrically determined enol contents and apparent absorptivities for a series of esters of acetoacetic acid in which the alkyl group of the ester was varied. The values of the absorptivities of the enols which may be calculated from their data for esters with primary and secondary alkyl groups

TABLE I

BROMOMETRIC AND ULTRAVIOLET ABSORPTION DATA ON ENOL CONTENTS OF 2-CARBOETHOXYCYCLANONES AND RELATED COMPOUNDS

Compound	% enol[a]	λ_{max}, mμ[b]	ϵ'_{max}[b]	ϵ_{enol} (calcd.)[c]
Cyclic β-keto esters				
I-5	6.3 ± 0.1[d]	255	677	10,800
I-6	59.1 ± 0.3[e]	258	6420	10,850
I-7	13.6 ± 0.2[f]	264	1625	11,900
I-8	42.4 ± 0.5[g]	262	4800	11,300
I-8	69.1 ± 0.5[h]	262[h]	7860[h]	11,400[h]
I-9	19.1 ± 0.1[g]	261	2190	11,500
I-10	48.7 ± 0.5[g]	261	5610	11,550
I-11	5.9 ± 0.1[g]	263	785	~13,000
I-12	3.3 ± 0.1[g]	261	426	~13,000
Acyclic β-keto esters				
Ethyl acetoacetate	13.6 ± 0.2[i]	244	1494	11,000
II	1.9 ± 0.1[j]	259	236	~12,000–13,000
III	0.4 ± 0.1[j]	~265	51	...
III	...	262[h]	150[h]	...
Ethyl β-alkoxycrotonates				
IV	99.0 ± 2	234	16.000	
IV	...	230[h]	15.700[h]	
V	98.0 ± 2	238	13,200	

[a] Unless otherwise noted, the enol contents were measured by the "modified" titrimetric method of Dieckmann[4] in 0.1 *M* ethanolic solutions which had been equilibrated at room temperature for at least 1 week. In all cases, the titrations were performed at least in duplicate; for most cases, the values given represent averages and average deviations of four or more determinations. [b] The values given are for equilibrated solutions in absolute ethanol at 25° unless noted otherwise. [c] The reliability of these values is estimated to be within ±400 units for those β-keto esters of enol contents of 10% or more. [d] The enol content of I-5 was checked by three methods: the Dieckmann method,[4] values 6.2, 6.4, and 6.3%; the indirect Meyer method,[10] values 6.4 and 6.5%; and the direct titration method,[5] values 6.2 and 6.1%. Other values reported for I-5 in ethanolic solution are 5[6] and 6.4%.[10] [e] Literature values for I-6 in ethanol are 57[6] and 60%.[4] [f] Literature values for I-7 in ethanolic solution are 12[6] and 14.0%.[4] [g] For the corresponding methyl esters in ethanol, Schwarzenbach and co-workers[6] reported: I-8, 40%; I-9, 15%; I-10, 50%; I-11, 9%; I-12, 5%. [h] These measurements refer to solution in cyclohexane; for titrimetric data, the concentrations were 0.1 *M* and equilibration times at least 1 week. [i] Measured by the method of Meyer.[10] Literature values, 13.2,[10] 11,[4] and 9–11%.[5] [j] Literature values of 3–4%[5] for II and 9–9.5[5] and 14%[8] for III are for samples containing varying amounts of IV and V, respectively. See footnote 7.

(4) W. Dieckmann, *Ber.*, **55**, 2477 (1922).

(5) J. B. Conant and A. F. Thompson, *J. Am. Chem. Soc.*, **54**, 4039 (1932).

(6) G. Schwarzenbach, M. Zimmerman, and V. Prelog, *Helv. Chim. Acta*, **34**, 1954 (1951).

(7) Special attention is directed to the values for these compounds since the values reported in the earlier literature[5,8] are undoubtedly too high owing to unsuspected contamination of the α-alkylated compounds with varying amounts of O-alkylated derivatives produced concurrently in their preparation. See S. J. Rhoads, R. W. Hasbrouck, C. Pryde, and R. W. Holder, *Tetrahedron Letters*, 669 (1963), and S. T. Yoffe, K. V. Vatsuro, E. E. Kugutcheva, and M. I. Kabachnik, *ibid.*, 593 (1965), for discussion of this point.

(8) M. I. Kabachnik, S. T. Yoffe, E. M. Popov, and K. V. Vatsuro, *Tetrahedron*, **12**, 76 (1961).

(9) In the course of this investigation, three established methods of bromine titration were tested: the direct method used by Conant and Thompson,[5] the indirect method of Meyer,[10] and the "modified" method of Dieckmann.[4] For the type of β-keto ester of concern here, the last method gave the most reproducible results although there was generally good agreement among the three. See footnote *d*, Table I.

(10) K. H. Meyer and P. Kappelmeier, *Ber.*, **44**, 2718 (1911).

(11) B. Eistert and W. Reiss, *Chem. Ber.*, **87**, 108 (1954).

(12) G. S. Hammond, W. G. Borduin, and G. A. Guter, *J. Am. Chem. Soc.*, **81**, 4682 (1959).

(13) F. Korte and F. Wüsten, *Ann.*, **647**, 18 (1961).

show a striking uniformity in the range 10,500 to 12,500 and are independent of the solvent medium. In view of the structural variety represented in the β-dicarbonyl systems mentioned above, it appears that those enolic species which exist as *cis*-chelated structures do exhibit a fairly constant value of absorptivity, which, moreover, is indifferent to the nature of the solvent. When precise measurements of enolic concentrations in such systems are prohibited by time or sample supply, estimates based on apparent absorptivities and an assumed value of 11,000–12,000 for the true absorptivity should not be grossly in error.

TABLE II

EQUILIBRATION STUDIES OF 2-CARBETHOXYCYCLOOCTANONE

Time	% enol (bromometric)	ϵ'(262 mμ)	ϵ_{enol} (calcd.)
	Solvent Ethanol		
1 hr.	69.6	7860	11,300
24 hr.	51.5	5910	11,500
48 hr.	46.7	5290	11,300
4 days	43.7	5130	11,700
7 days	...	5030	...
14 days	42.4	4800[a]	11,300
	Solvent Cyclohexane		
24 hr.	76.0	9080	11,900
4 days	73.5	8360	11,400
9 days	71.7	8270	11,500
14 days	69.1	7860[a]	11,400

[a] Equilibrium value.

Eistert and Reiss[11] have pointed out an important and useful relationship between the spectral characteristics of enols of β-dicarbonyl compounds and those of their corresponding ethers. For "*trans*-fixed" enols, a conversion of the enol to its enol ether results in little or no shift in the absorption maximum and, generally, an appreciable *decrease* in the absorptivity in a given solvent. Thus, dimedone has a characteristic enolic absorption at 251 mμ in methanol with an absorptivity value of 16,700 whereas its methyl enol ether shows a maximum at the same wave length in methanol with an absorptivity of 14,600. On the other hand, the conversion of a *cis*-chelated enol of an open-chain β-keto ester to a *trans* ether is accompanied by a distinct *hypsochromic* shift and, usually, by an appreciable *increase* in the absorptivity.[11,14] This is illustrated in Table I by a comparison of the positions of the maxima and the absorptivity values of the *trans* enol ethers IV and V[15] with those of their enolic parent, ethyl acetoacetate. These facts serve to emphasize the error involved in the practice of using absorptivity values of enol ethers as limiting values for the absorptivity of the *cis*-enolic species itself.

For many of the β-keto esters studied in this investigation, it was found that equilibrium between the tautomers was established very slowly. This was especially true for those β-keto esters of low acidity, *i.e.*, acyclic α-substituted acetoacetates and cyclic keto esters of ring members ≧7. In experiments with 2-carbethoxycyclooctanone (I-8), summarized in Table II, we have followed these changes in enol content both bromometrically and spectrometrically and have found that as much as 2 weeks may be required before the enol content ceases to change. These final values, representing true equilibrium, may, of course, be attained more or less rapidly according to the purity of the solvents and the purity and history of the sample. In the experiments shown in Table II, the sample used was one of high purity, which, following its final purification by fractional distillation, had been stored neat at −30°.

Experimental

Materials.—All solvents employed in this study were of spectral quality. The preparation and purification of the cyclic β-keto esters I-*n* and the acyclic compounds II and III have been described elsewhere.[3,7] It is important to note that II and III were completely freed of contaminating O derivatives by treatment with dilute hydrochloric acid[7] prior to final work-up. The purities of the samples of II and III used in these studies were established as >99% by g.l.p.c.

Ethyl β-ethoxycrotonate (IV) was prepared by the method of Michael and Carlson[16] from ethyl acetoacetate, ethyl orthoformate, and ferric chloride, b.p. 76–77° (7 mm.), m.p. 30.5–30.8° cor., purity by g.l.p.c. >99%.

Ethyl β-*sec*-butoxycrotonate (V) was prepared from ethyl β-chlorocrotonate[17] and sodium *sec*-butoxide in *sec*-butyl alcohol. A heart cut of the evaporatively distilled product was demonstrated by g.l.p.c. and n.m.r. analysis to consist of 80% V and 20% of the transesterified product, *sec*-butyl β-*sec*-butoxycrotonate.[18] The values given for V in Table I are therefore corrected for this contamination.

Titration Procedure.[4]—Solutions 0.1 *M* in β-keto ester in absolute ethanol or in cyclohexane were equilibrated at room temperature in the dark for 1 or 2 weeks. After this time, 10- or 25-ml. aliquots were withdrawn and cooled to −30° preparatory to titration. (At the same time appropriate portions of the stock solution were diluted for the ultraviolet measurements which were made immediately.) To the chilled solution of β-keto ester was added a 10-ml. portion of a cold (−30°), methanolic solution of bromine. The concentration of the bromine solution varied from *ca.* 0.5 to 0.05 *M* according to the enol content and sample size of the ester titrated. Immediately after the addition of the bromine solution, a solution of 1–2 g. of potassium iodide in 5 ml. of water was added and the released iodine was titrated with standard 0.1 *N* thiosulfate solution. A blank was run on another 10-ml. portion of the cold bromine solution immediately following the titration of the ester. Magnetic stirring was used throughout the procedure and all operations were carried out as rapidly as possible. Titrations of the enol ethers IV and V were carried out in the same manner, except that the equilibration time was omitted. For those β-keto esters of relatively low enol content or for those available in only limited amounts, the method was adjusted to a semimicro scale by employing the more dilute bromine solution, 0.025 *N* thiosulfate, and a microburet.

Ultraviolet Studies.—Absorptivity measurements were made in matched 1-cm. silica cells with a Beckman DB spectrophotometer in which the cell compartment was maintained at 25°.

(14) In general, these same changes will not be so marked or so predictable with the conversion of the *cis*-chelated enol to the *cis*-enol ether. Although Eistert and Reiss found that the *cis*-methyl enol ether of acetylacetone showed a maximum 10 mμ toward shorter wave length than acetylacetone itself and an absorptivity some 2000 units greater, we have found that the (necessarily *cis*) methyl ether of I-5 absorbs at the same wave length as its parent and with an absorptivity only 1000 units greater. With increasing ring size, which produces increasing steric pressures between the oxygenated functions in the *cis*-enol ethers, the absorptivity of the ether falls below that of the enol but a hypsochromic shift again appears: R. W. Hasbrouck, Ph.D. Dissertation, University of Wyoming, 1964.

(15) The *trans* configurations of IV and V are firmly established by their infrared and n.m.r. spectral characteristics: unpublished work, this laboratory.

(16) A. Michael and G. H. Carlson, *J. Am. Chem. Soc.*, **57**, 159 (1935).

(17) D. E. Jones, R. O. Morris, C. A. Vernon, and R. F. White, *J. Chem. Soc.*, 2349 (1960).

(18) Unpublished experiments of R. W. Hasbrouck. We thank Dr. Hasbrouck for supplying us with this sample.