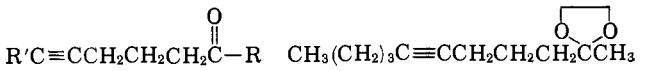
In the second sequence propargylacetone[4] was converted in 80% yield into its cyclic ketal (VI),[5] b.p. 75–76° (15 mm.), and this was then alkylated (sodamide–liquid ammonia) with *n*-amyl bromide to give VII, identical with the product from the first route.

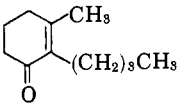
When the acetylenic ketone (V) was hydrated by refluxing with hot aqueous methanolic sulfuric acid in the presence of mercuric sulfate *exclusive formation of the 1,4-diketone*, the known 2,5-undecanedione[6] (IV, R′ = CH_3; R = $CH_3(CH_2)_4$) was observed, b.p. 80–85° (0.1 mm.), 85% yield. The structure of the 1,4-dione was proved by its almost quantitative cyclization to dihydrojasmone (2-*n*-amyl-3-methyl-Δ^2-cyclopentenone-VIII), b.p. 79–81° (0.2 mm.), single peak on gas chromatography (20% SE-30 at 175°), λ_{max}^{EtOH} 237 mμ (ε 12,000); further identified as its semicarbazone, m.p. 175–176° as reported.[7]

The exclusive formation of a 1,4-diketone requires carbonyl participation in the hydration step *via* a kinetically and geometrically favored five-membered ring. Participation *via* a six-membered ring should however be possible in acetylenic ketones of type IX, thus *leading specifically to 1,5-diketones X*. This turned out also to be the case: Alkylation of the cyclic ketal of 6-heptyn-2-one (made by alkylation of sodium

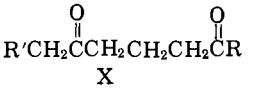


IX

XI



XII



X

acetylide in liquid ammonia with the dioxolane of 3-bromopropyl methyl ketone) with butyl iodide, using sodamide in liquid ammonia, gave the corresponding disubstituted acetylene (XI) in 60% yield. Deketalization with aqueous perchloric acid at room temperature led to the required 6-undecyn-2-one (IX, R = CH_3; R′ = $CH_3(CH_2)_3$) in 93% yield, b.p. 85–87° (0.3 mm.). The acetylenic ketone was transformed on refluxing with aqueous methanolic sulfuric acid and mercuric sulfate into a single diketone (one peak on 20% Craig column at 190°), the structure of which as 2,6-undecanedione (X, R = CH_3; R′ = $CH_3(CH_2)_3$) was confirmed by its base-catalyzed cyclization to 2-butyl-3-methyl-Δ^2-cyclohexenone (XII, 82% yield from the acetylenic ketone), b.p. 63–65° (0.2 mm.), λ_{max}^{EtOH} 242 mμ (ε 13,400), only one peak on gas chromatography (Craig, 190°), 2,4-dinitrophenylhydrazone m.p. 142–143° as reported.[8]

Specific hydration of acetylenes to 1,5-diketones *via* δ-carbonyl participation can thus also take place, but it is significant that this hydration is, as expected, markedly slower than with the γ-carbonyl analog. This is shown by the fact that it is possible to define conditions which will result in complete hydration of γ-ketoacetylenes while leaving the δ-keto compounds unchanged.

(4) J. Colonge and R. Gelin, *Bull. soc. chim. France*, 208 (1954).

(5) A different synthesis of this ketal has recently been published by C. Feugeas, *ibid.*, 2579 (1963).

(6) H. Hunsdiecker, *Ber.*, **75**, 447 (1942).

(7) W. Tieff and H. Werner, *ibid.*, **68**, 640 (1935).

(8) A. J. B. Edgar, J. H. Harper, and M. A. Kazi, *J. Chem. Soc.*, 1083 (1957).

(9) Predoctoral Fellow, National Institutes of Health.

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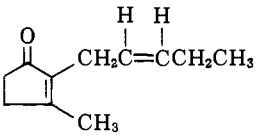
RICHARD BORCH[9]

RECEIVED JANUARY 28, 1964

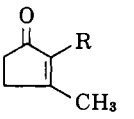
A Synthesis of *cis*-Jasmone

Sir:

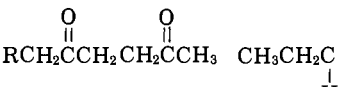
It was demonstrated by Hunsdiecker[1] that 2-alkyl 3-methyl-Δ^2-cyclopentenones (I) are readily prepared by base-catalyzed cyclization of 1,4-diketones of the general formula II, which were themselves available by a variety of classical methods. The discovery in our laboratory of a new synthesis of 1,4-diketones[2] suggested the possibility of its use for the preparation of diketone III, the precursor of the well-known jasmone (IV), the important constituent of the essential oil of jasmine.[3]



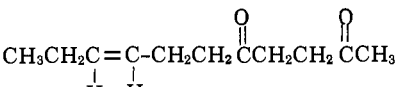
IV



I



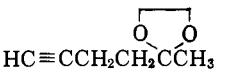
II



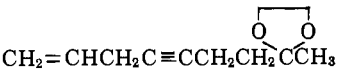
III

We first demonstrated the feasibility of the synthesis for the preparation of 3-methyl-Δ^2-cyclopentenones with an unsaturated side chain in the 2-position by synthesizing I (R = allyl).

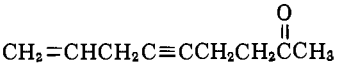
Alkylation of the magnesium halide complex of V with allyl chloride in the presence of cuprous chloride led to cyclic ketal VI obtained in 61% yield (b.p. 78–81° at 0.5 mm.) and deketalized (91%) as usual to VII, b.p. 70–77° (0.5 mm.), semicarbazone m.p. 99–101°. Hydration in the standard manner gave 83% of 8-nonene-2,5-dione (II, R = allyl), b.p. 75–77° (0.5 mm.), cyclized in 93% yield to the known 2-allyl 3-methyl-Δ^2-cyclopentenone (I, R = allyl), b.p. 71–73°, λ_{max}^{EtOH} 236 mμ (ε 12,000), 2,4-dinitrophenylhydrazone m.p. 174–175°.[4]



V



VI



VII

In the synthesis of diketone III, the precursor of jasmone, two problems were faced which are absent with the simple allyl derivative just described.

The first of these was the occurrence of rearrangements, which we could not suppress, in the formation of *cis*-1-halo 2-pentene. This was solved when it was found that *cis*-2-pentene-1-ol[5] (VIII) could be transformed in 95% yield, upon treatment with toluenesulfonyl chloride and powdered sodium hydroxide in the cold, into a crude tosylate which was satisfactory for use in the alkylation of V. In that alkylation, the use of the magnesium complex of V proved unsatisfactory, but success was achieved by the use of the lithium salt of V in liquid ammonia which gave ketal IX in 45% yield, b.p. 100–103°. Deketalization of IX with aqueous perchloric acid (94% yield) at room

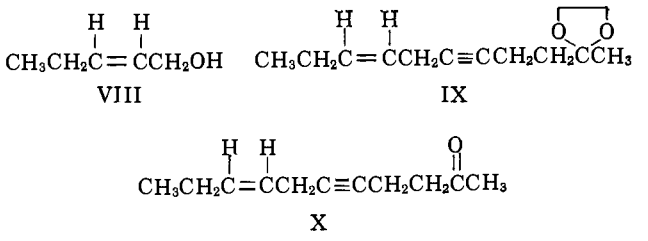
(1) H. Hunsdiecker, *Ber.*, **75**, 447 (1942).

(2) G. Stork and R. Borch, *J. Am. Chem. Soc.*, **86**, 935 (1964).

(3) For a review see E. L. Saul, *Am. Perfumer*, **45**, 27 (1943).

(4) L. Crombie, A. J. B. Edgar, S. H. Harper, M. W. Lowe, and D. Thompson, *J. Chem. Soc.*, 3552 (1950).

(5) *Cf.* J. Colonge and J. Poilane, *Bull. soc. chim. France*, 953 (1955).



VIII IX

X

temperature gave pure *cis*-8-undecen-5-yn-2-one (X), b.p. 81–84° (0.2 mm.), one peak on v.p.c. (20% Craig, 215°), no ultraviolet absorption, and no *trans* double bond peak at 10.3 μ. The *p*-nitrophenylhydrazone had m.p. 95–96°.

The second problem came from the fact that it was now necessary to hydrate the acetylenic link in X without affecting the *cis* double bond. Here the remarkable acceleration of the rate of hydrolysis by γ-carbonyl participation became crucially important. Whereas the usual hydration conditions (hot aqueous methanolic sulfuric acid and mercuric ion) led to almost complete *cis–trans* isomerization, it was possible to effect the desired reaction without any involvement of the *cis* double bond by keeping X at room temperature for 1.5 hr. with dilute aqueous methanolic sulfuric acid–mercuric sulfate.[6] The resulting 1,4-diketone III was then cyclized with dilute aqueous base, in the usual manner, to jasmone (75% over-all from ketal IX). This showed only one peak on v.p.c. (20% Craig, 220°) and was identified as *cis*-jasmone by its ultraviolet spectrum [λ_{max}^{EtOH} 237 mμ (ϵ 11,000)], the identity of its infrared spectrum with that of authentic material,[7] and by its 2,4-dinitrophenylhydrazone, m.p. 117.5° as reported.[7]

(6) Under these conditions, the δ-ketoacetylenes are completely unchanged.

(7) L. Crombie and S. H. Harper, *J. Chem. Soc.*, 869 (1952).

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RICHARD BORCH

RECEIVED JANUARY 28, 1964

The Observation of Acyl–Enzyme Intermediates in the α-Chymotrypsin-Catalyzed Hydrolysis of Specific Ester Substrates at Low pH[1]

Sir:

The α-chymotrypsin-catalyzed hydrolyses of the ethyl, methyl, and *p*-nitrophenyl esters of N-acetyl-L-tryptophan were shown by means of an indirect kinetic argument to proceed through the formation of a common N-acetyl-L-tryptophanyl-α-chymotrypsin intermediate.[2] For the methyl ester, the half-lives at pH 7 for the formation, k_2, and decomposition, k_3, of this intermediate (eq. 1) were calculated to be 1 and 30 msec., respectively, too fast for ordinary or even most stopped-flow instrumentation to measure directly. However, both k_2 and k_3 are dependent on

$$E + S \overset{K_m}{\rightleftarrows} ES \xrightarrow{k_2} ES' \xrightarrow{k_3} E + P_2 \qquad (1)$$
$$+ P_1$$

a basic group with a pK_a of *ca.* 7.[3] Therefore, at pH 3, the half-lives of the above individual steps should be of the order of seconds rather than milliseconds, and thus amenable to direct measurement.

(1) This research was supported by grants from the National Institutes of Health, part XXV in the series: The Mechanism of Action of Proteolytic Enzymes.

(2) B. Zerner and M. L. Bender, *J. Am. Chem. Soc.*, **85**, 356 (1963).

(3) M. L. Bender, G. E. Clement, F. J. Kézdy, and B. Zerner, *ibid.*, **85**, 358 (1963).

At pH 7, the catalytic rate constants (of the turnover, $k_2k_3/(k_2 + k_3)$) of the α-chymotrypsin-catalyzed hydrolysis of the ethyl and *p*-nitrophenyl esters of N-acetyl-L-tryptophan were shown to be equivalent to one another.[2] The catalytic rate constants of these two reactions are essentially identical from pH 7 down to pH 2 (see Fig. 1). Since the enzyme contains many carboxylate ions, the protonation of these groups should electrostatically perturb the ionization constant of the basic group of pK_a = 7. Thus, whereas ionic strength effects on the rate are negligible near neutrality, they are important at low pH because of the large positive charge on the enzyme.[4] The intrinsic pK_a of the group, on which both these ester hydrolyses are dependent, is 7.2, using data from pH 2 to 7. Furthermore, titration of the concentration of enzymatic active sites by N-*trans*-cinnamoylimidazole around neutrality is exactly equivalent to titration of that same solution by the burst of *p*-nitrophenol in the reaction of N-acetyl-L-tryptophan *p*-nitrophenyl ester[5] at pH 2.3, using pure α-chymotrypsin. Thus the relative rates of reaction, titration of active sites, and the pH dependence of the enzyme appear to be normal as low as pH 2, and thus it is feasible to carry out mechanistic investigations at low pH.[6]

The object of such investigations is to spectrophotometrically observe the individual steps of eq. 1, which are not discretely observable at pH 7. The α-chymotrypsin-catalyzed hydrolysis of N-acetyl-L-tryptophan *p*-nitrophenyl ester at pH 2.3 shows a typical initial burst followed by a steady-state production of *p*-nitrophenol. This observation can only be interpreted in terms of the rapid formation and slow decomposition of an N-acetyl-L-tryptophanyl-α-chymotrypsin intermediate.[7] The formation of this intermediate is stoichiometric, as mentioned above, by comparison with titration of the enzymatic sites with N-*trans*-cinnamoylimidazole.

The technique of the observation of the acyl–enzyme in the hydrolysis of N-acetyl-L-tryptophan methyl ester consisted in spectrophotometrically observing the time course of the reaction under conditions of $(E)_0 > (S)_0$ and $(E)_0 > K_m$, so that two consecutive first-order processes are observed. When the substrate is saturated with enzyme [*sic*] and when the enzyme is in greater concentration than the substrate, the initial first-order process measures k_2 and the final first-order reaction measures k_3 (if $k_2 > k_3$).[8] These are approximately the conditions[9] under which the α-chymotrypsin-catalyzed hydrolysis of N-acetyl-L-tryptophan methyl ester was carried out at pH values of 2.3, 3.4, and 4.3. A typical spectrophotometric experiment is shown in Fig. 1 of the accompanying communication.[10] In each experiment, the initial absorbance of the ester decreased rapidly, reached a minimum, and then slowly rose, reaching an infinity

(4) Evidence for a functional carboxyl group [J. A. Stewart, H. S. Lee, and J. E. Dobson, *ibid.*, **85**, 1537 (1963)] is probably related to these large ionic strength effects.

(5) The DL-ester was used at an $(E)_0/(S)_0$ ratio high enough to obviate the side reaction of the D-compound noted earlier.[2]

(6) The enzyme is stable at pH values of 2 to 3: in fact pH 2 is often used to crystallize α-chymotrypsin [M. Laskowski, "Methods in Enzymology," Vol. 2, S. P. Colowick and N. O. Kaplan, Ed., Academic Press, New York, N. Y., 1955, p. 12].

(7) H. Gutfreund and B. R. Hammond [*Biochem. J.*, **73**, 526 (1959)] report a similar phenomenon in the α-chymotrypsin-catalyzed hydrolysis of N-benzyloxycarbonyl-L-tyrosine *p*-nitrophenyl ester at pH 7.2, but do not indicate that the reaction is stoichiometric.

(8) M. L. Bender and B. Zerner, *J. Am. Chem. Soc.*, **84**, 2550 (1962); F. J. Kézdy and M. L. Bender, *Biochem.*, **1**, 1097 (1962).

(9) Although the requirement of $(E)_0 > (S)_0$ was always obeyed, the requirement of $(E)_0 > K_m$ is problematical because of experimental limitations so that some rate constants may not be true maximal rate constants at saturation conditions.

(10) F. J. Kézdy and M. L. Bender, *J. Am. Chem. Soc.*, **86**, 938 (1964).