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Photochemistry of Three *N*-Acetoacetyl Amino Acid Methyl Esters: Structure Elucidation of the Radiation Products by Gas Chromatography/Mass Spectrometry

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The photochemistry of three *N*-acetoacetyl α-amino acid (valine, *tert*-leucine, isoleucine) methyl esters was investigated. The products after UV irradiation in acetonitrile at 300 nm (direct excitation) were analyzed by gas chromatography coupled with chemical ionization mass spectrometry. In all cases a complex product mixture was found as the result of the nπ* excitation of the substrates. The major reaction paths were Norrish type I reactions and hydrogen atom abstractions with concomitant radical cleavage and radical recombination steps. © **1998 John Wiley & Sons, Ltd.**

KEYWORDS: photochemistry; α-amino acid methyl esters; gas chromatography/chemical ionization mass spectrometry

INTRODUCTION

In a long-term research project, we are investigating the photochemical behavior of *N*- and *C*-activated amino acids and peptide model substrates.[1,2] The background of these studies is the understanding of energy and electron transfer processes in oligopeptides. In order to correlate short- and long-distance processes with the properties of the excited part of molecule, we have been systematically investigating model compounds with restricted reactive sites. The *N*-acetoacetyl α-amino acid methyl esters **1a–c** used in this study were expected to exhibit preferentially triplet nπ* carbonyl photochemistry. The preferred reaction paths for these chromophores are Norrish type I (α-C—C homolysis) and Norrish type II reactions (γ-hydrogen atom abstraction). The latter process was not feasible for substrates **1** because of the strong NγH bond. We investigated the complex product mixtures from direct photolysis in acetonitrile using gas chromatography/mass spectrometry (GC/MS).

EXPERIMENTAL

Synthesis of substrates 1a–c

To a cooled solution (0 °C) of 10 mmol of the hydrochloride salt of the amino acid methyl ester in 5 ml of aqueous 1.75 M sodium carbonate solution, 10 mmol of diketene were added over a period of 30 min. After stirring at room temperature for 16 h, the solvent was evaporated under reduced pressure and the residue was extracted with 100 ml of CH_2Cl_2. After treatment with 80 ml of water, the organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure. Compounds **1a–c** were obtained as a yellow oil.

Irradiation procedure

A solution (9.3 mmol l^{-1}) of **1a–c** in acetonitrile in a Pyrex vessel purged with a constant stream of dry nitrogen was irradiated for 15 h in a Rayonet RPR-208 photochemical reactor equipped with eight fluorescence lamps (300 ± 5 nm, *ca.* 800 W). The crude product mixtures were analyzed directly by GC/MS and by NMR spectroscopy.

Instrumentation

All GC/CIMS measurements were performed with a Finnigan Incos 500 quadrupole mass spectrometer (Finnigan MAT, San Jose, CA, USA) coupled with a Varian Model 3400 gas chromatograph (Varian Analytical Instruments, Sunnyvale, CA, USA) with a split/splitless injector and an Optima-5 capillary column (25 m × 0.25 mm i.d., 0.25 μm film thickness) (Macherey–Nagel, Düren, Germany). The helium flow-rate was 1 ml min^{-1} (head pressure 55 kPa). Injection (1 μl) was performed manually, with a split ratio of 10:1 and an injection temperature of 250 °C. The oven temperature program was initial temperature 60 °C, held for 1 min, then increased at 10 °C min^{-1} to 250 °C, which was held for 5 min. The transfer line temperature was 250 °C. The Incos 500 was equipped with ion sources

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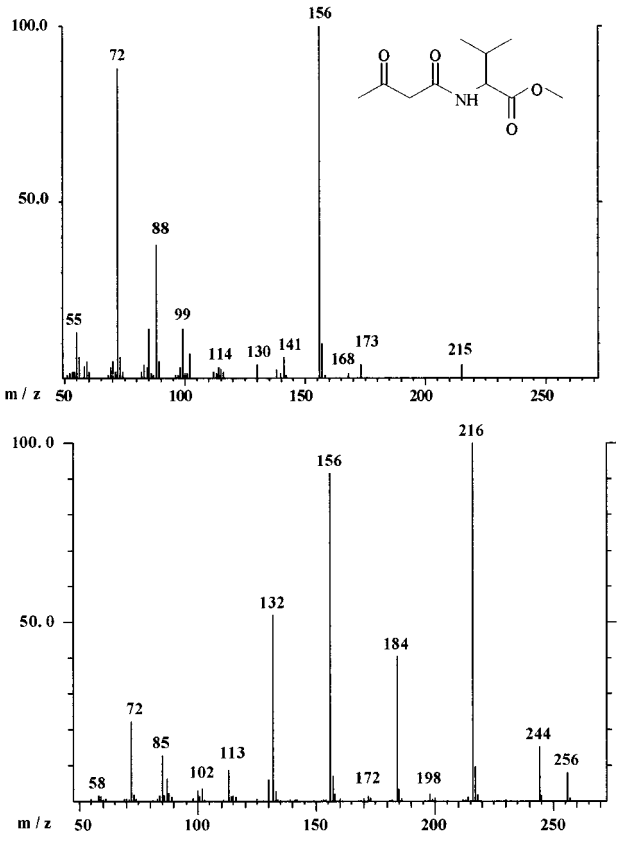


Figure 1. Mass spectra of *N*-acetoacetylvaline methyl ester. Top, EI (70 eV); bottom, CI(CH_4). $M_r = 215$.

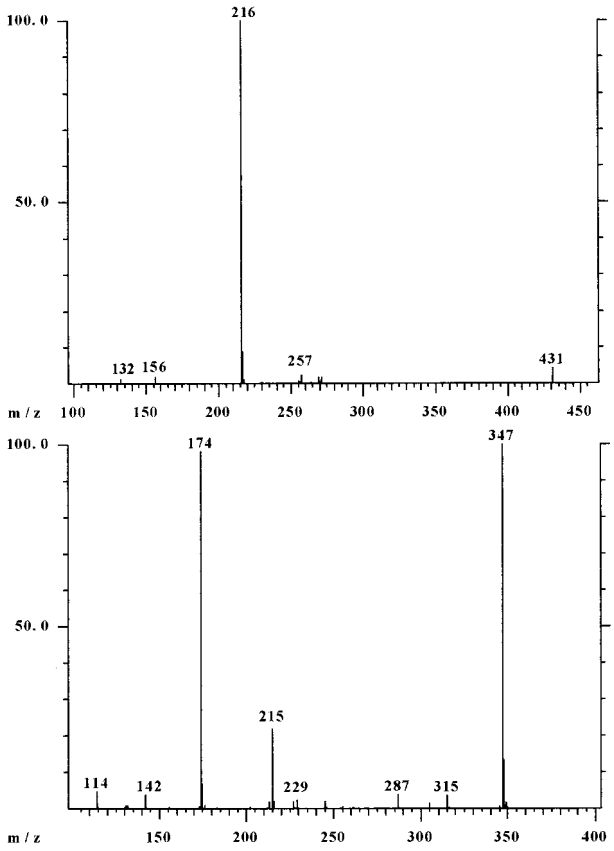


Figure 2. CI(CH_3CN) mass spectra of *N*-acetoacetylvaline methyl ester (top, $M_r = 215$) and *N*-acetylvaline methyl ester (bottom, $M_r = 173$).

for CI and EI (70 eV). MS parameters were: ion source set temperature 100 °C for CI and 180 °C for EI, electron multiplier voltage 1400 V (CI) and 1150 V (EI). GC/MS data were acquired using Data General DG-20.

RESULTS

For these investigations we used electron ionization (EI) and CI with methane [CI(CH_4)] and acetonitrile [CI(CH_3CN)]. EI, CI(CH_4) and CI(CH_3CN) mass spectra of *N*-acetylvaline methyl ester and *N*-acetoacetylvaline methyl ester are compared in Figs 1 and 2. As can be seen, the EI mass spectra of these compounds (e.g. *N*-acetoacetylvaline methyl ester, Fig. 1) are characteristic, but the molecular ions cannot be recognized with certainty. Acetonitrile as CI reagent gas has the advantage of not being corrosive towards metal surfaces (as, e.g., NH_3). It has a high proton affinity

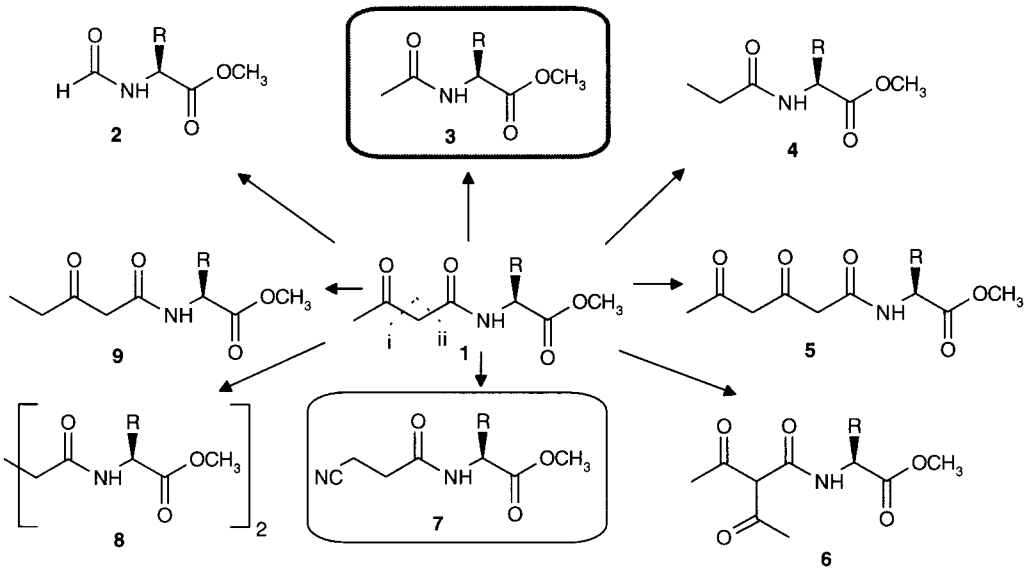


Figure 3. *N*-Acetoacetyl amino acid esters (**1**) and photochemically generated products **2**–**9**. Amino acids: **a**, valine (R = *iso*-C_3H_7); **b**, *tert*-leucine (R = *tert*-C_4H_9); **c**, isoleucine [R = $CH(CH_3)CH_2CH_3$].

Table 1. CI(CH_4) mass spectra of 1–9: *m/z* (relative intensity, % to base peak)

Compound (%)[a]	MH^+ [b]	$[MH - HCO_2CH_3]^+$	$[MH - CH_3OH]^+$	$[MH - nCH_2CO]^+$ $n=1$	$n=2$	$n=3$	*m/z*[h]
1a (5.0)	**216**[c]	156 (92)[d]	184 (42)	—	132 (53)	—	72 (23)
2a (<0.3)	160 (97)	**100**	128 (30)	—	—	—	
3a (68)	**174**	114 (78)	142 (67)	132 (32)	—	—	72 (7)
4a (5.0)	**188**	128 (45)	156 (38)	132 (32)[e]	—	—	72 (7)
5a (1.4)	**258**	198 (10)	226 (8)	216 (35)	174 (66)	132 (53)	156 (22)
6a (1.6)	285 (20)	198 (5)	226 (3)	**216**	174 (8)	132 (24)	156 (11)
7a (9.7)	**213**	153 (78)	181 (39)	—	—	—	72 (3)
8a (8.7)	345 (95)	285 (8)	313 (14)	132 (11)[f]	—	—	**214**
9a (<0.3)	**230**	170 (38)	198 (24)	—	132 (67)[g]	—	99 (46)
1b (7.1)	**230**	170 (82)	198 (37)	—	146 (31)	—	86 (18)
2b (1.5)	174 (90)	**114**	142 (67)	—	—	—	
3b (65)	**188**	128 (73)	156 (58)	146 (9)	—	—	86 (4)
4b (3.0)	**202**	142 (43)	170 (37)	146 (10)[e]	—	—	86 (4)
5b (1.7)	**272**	212 (8)	240 (10)	230 (27)	188 (38)	146 (44)	170 (14)
6b (2.2)	272 (27)	212 (3)	240 (2)	**230**	188 (5)	146 (19)	170 (9)
7b (11.0)	227 (87)	**167**	195 (54)	—	—	—	86 (4)
8b (8.0)	373 (98)	313 (3)	341 (15)	146 (15)[f]	—	—	**228**
9b (0.5)	**244**	184 (48)	212 (37)	—	146 (44)[g]	—	99 (33)
1c (<0.3)	**230**	170 (62)	198 (24)	—	146 (49)	—	86 (50)
2c (3.4)	**174**	114 (80)	142 (32)	—	—	—	
3c (65)	**188**	128 (51)	156 (35)	146 (13)	—	—	86 (4)
4c (5.9)	**202**	142 (35)	170 (17)	146 (18)[e]	—	—	86 (6)
5c (2.3)	**272**	212 (9)	240 (6)	230 (37)	188 (53)	146 (28)	170 (10)
6c (4.5)	272 (23)	212 (4)	240 (2)	**230**	188 (15)	146 (25)	170 (13)
7c (11.2)	**227**	167 (67)	195 (37)	—	—	—	86 (3)
8c (7.1)	**373**	313 (2)	341 (11)	146 (23)[f]	—	—	228 (69)
9c (<0.3)	244 (78)	184 (18)	212 (20)		146 (11)[g]	—	99 (96)

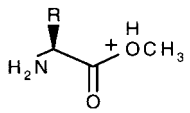
[a] By GC/MS; normalized GC peak areas in % after the irradiation for 15 h in acetonitrile at 300 nm and 35 °C (the initial concentration of **1** was 9.3 mmol l^{-1}).
[b] Molecular masses were determined from $[M + H]^+$, $[M + C_2H_5]^+$ and $[M + C_3H_5]^+$ ions in the CI(CH_4) spectra.
[c] The most abundant ion is given in bold type.
[d] *m/z* (relative intensity, %).
[e] $[M - CH_3CHCO]^+$.
[f] $[H_2NCH(R)COOCH_3 + H]^+$.
[g] $[MH - CH_3CH_2COCHCO]^+$.
[h] Other characteristic peaks.

(*PA*) of 782 kJ mol^{-1}, which lies between those of methane (531 kJ mol^{-1}) and isobutane (816 kJ mol^{-1}).[3–5] We successfully tested CI(CH_3CN) for some amino acid derivatives (e.g. *N*-acetoacetyl- and *N*-acetylvaline methyl ester, Fig. 2). The protonated molecular ions, cluster ions $[MH + CH_3CN]^+$ and dimer cluster ions $[2M + H]^+$ were found to be the most abundant species. The essential absence of fragment ions permits no further structure information to be derived. In contrast, CI(CH_4) produces protonated molecules, $[M + C_2H_5]^+$ and $[M + C_3H_5]^+$, and also characteristic fragment ions[6] (Table 1).

The substrates **1a–c** and the photochemically generated products **2–9** detected in this GC/MS study are shown in the Fig. 3. The main products under standard irradiation conditions (the initial concentration of **1** was 9.3 mmol ml^{-1}) were the Norrish type I cleavage products **3**. The formation of the precursor radical $^{\cdot}CH_2CONHCH(R)COOCH_3$ is evident because of the formation of the products **4**, **7** and **8** due to competing pathways. Substances **5** and **6** originate from radical acetylation at the β- and δ-positions, respectively (see below).

CI(CH_4) mass spectra

The mass spectra obtained by GC/MS are presented in Table 1. The molecular masses were determined from the characteristic peaks of the protonated molecules MH^+ and the ions $[M + C_2H_5]^+$ and $[M + C_3H_5]^+$. The ester function was readily identified by the elimination of CH_3OH, $[MH - 32]^+$, and HCO_2CH_3, $[MH - 60]^+$, from the protonated molecules.[7,8] The various substituents at the *N*-termini gave rise to ion **a** (*m/z* 132 and 146 for valine and leucine derivatives, respectively).



a, R = C_3H_7 or C_4H_9

The elimination of $(CH_2CO)_n$ from the MH^+ ion is in accordance with the *N*-termini of compounds **1**, **3**, **5**, and **6**. The structural differences of isomers **5** and **6** are readily recognized in the CI(CH_4) spectra, while the EI

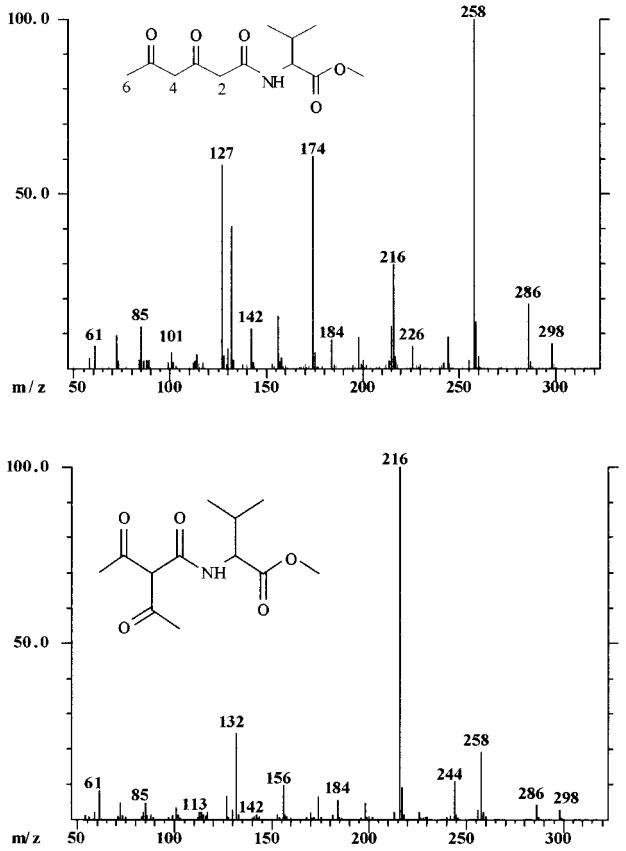
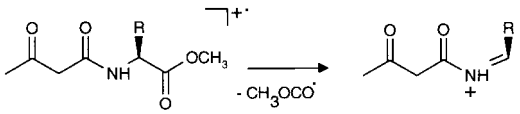


Figure 4. CI(CH_4) mass spectra of two isomeric photochemically generated products **5a** (top) and **6a** (bottom).

and CI(CH_3CN) spectra are similar (see, for example, the spectra of **5a** and **6a** in Fig. 4).

DISCUSSION

The predominant primary process for substrates **1a–c** after electronic excitation and intersystem crossing to the triplet excited state was the cleavage of the α-(C=O)—C bond with formation of an acyl radical and a primary carbon radical (dashed line i in Fig. 3). This clearly shows that the excitation energy is preferentially localized in the ketone carbonyl group and not in the amide carbonyl group. After homolysis, the radical pair underwent a multitude of secondary reactions. Hydrogen atom transfer to give the *N*-acetyl amino acid esters **3a–c** prevailed (*ca.* 70% in the original reaction mixture). A less favorable path was coupling with an acetonitrile radical to give the products **7a–c**. The competing dimerization of the primarily formed precursor radical $^{\cdot}CH_2CONHCH(R)COOCH_3$ led to the compounds **8a–c**. For the substrates **1a–c** traces of the *N*-formyl compounds **2a–c** were found as secondary products of the alternative Norrish type I process α-(O=CNH)—C bond cleavage; dashed line ii in Fig. 3). The other minor products **4**, **5**, **6** and **9** derived from complex coupling processes with a methyl or an acetyl radical, respectively. Finally, it is remarkable that both the alkyl substituent R and the ester group remained intact even after prolonged irradiation. Comparison of the photochemistry of *N*-acetoacetyl amino acid methyl esters **1a–c** (at $\lambda_{ex.} = 300$ nm in acetonitrile) with that of the *N*-acetyl amino acid methyl esters (at $\lambda_{ex.} = 254$ nm in methanol) revealed that the latter compounds are efficiently degraded by α-cleavage of the $COOCH_3$ group and, less efficiently, by α-cleavage of the alkyl group (exemplified for the valine derivative in Table 2[8]). The analogous process was found to be characteristic also in EI-induced fragmentation in both *N*-acetyl and *N*-acetoacetyl amino acid methyl esters.



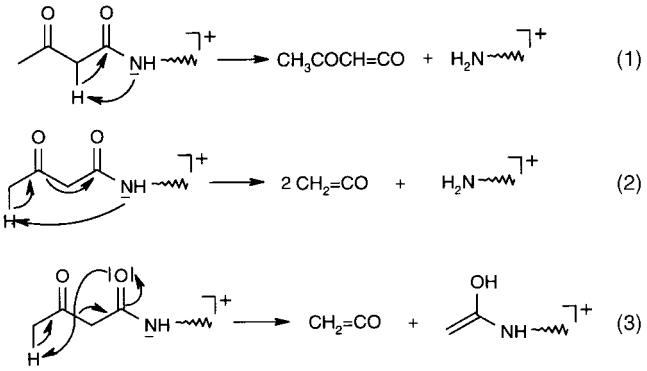
In contrast to EI, the characteristic fragmentations under CI(CH_4) conditions are the elimination of methanol and of the protonated methoxycarbonyl group. The most interesting feature is the elimination of one to three CH_2CO units from **1**, **3**, **5** and **6** (and accordingly of $C_2H_5COCH_2CO$—H from **9**). Loss of CH_2CO from $[M + H]^+$ of *N*-acetyl amino acids is well documented.[9] For the degradation of the acetoacetyl group, three mechanisms can be envisaged, as shown in Eqns (1)–(3).

Process (1) corresponds to the loss of ketene from **3** and (2) represents an analogous H-transfer via a six-membered transition state. Both result in the loss of the entire acyl group, whereas (3) leads to the loss of CH_2CO, and an enol is formed which would not be expected to fragment by a further loss of CH_2CO. The

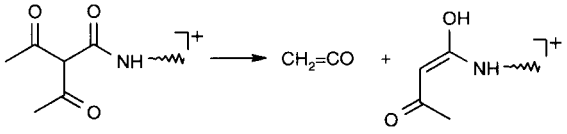
Table 2. Elimination processes of *N*-acetylvaline methyl ester under EI, CI(CH_4) and photodissociation (254 nm, in CH_3OH)

Process	*m/z* (% to base peak) EI (70 eV)	*m/z* (% to base peak) CI(CH_4)	Photochemical product, molecular mass
$M - COOCH_3$ (α-cleavage)	114 (90)	114 (95–100)	115[a]
$M - CH_3O$	142 (3)[b]	142 (80; $[MH - CH_3OH]^+$)	141[c]
$M - CH_2CO$	131 (9)	132 (40; $[MH - CH_2CO]^+$)	—
$M - C_3H_7$ (α-cleavage)	130 (3)[c]	—	131

[a] Main product after irradiation for 9 h in CH_3OH at 254 nm; the probable mechanism is the formation of the precursor radical $AcNHCH^{\cdot}CH(CH_3)_2$ followed by proton association.
[b] Takes place simultaneously with elimination of CH_2CO (*m/z* 99).
[c] Four minor products.



observation that **1a–c** show a loss of $(CH_2CO)_2$ but not of CH_2CO suggests that mechanism (1) or (2) is operating. These considerations are useful for an explanation of the differences in the fragmentation patterns of **5** and **6**. Compound **5** shows a pronounced loss of one, two and three CH_2CO molecules, whereas for **6** the loss of $(CH_2CO)_2$ is drastically reduced and that of one CH_2CO dominates the spectrum. Mechanisms (1) and (2) lead in either case to the elimination of $(CH_2CO)_3$;



for **6** it may be less favorable for steric reasons. Mechanism (3) explains the loss of the one and two CH_2CO units (transfer of H from C-6 or C-4; see Fig. 4) from **5**, whereas from **6** only one CH_2CO will be lost. Hence the assignment of the respective structures to the spectra is reasonable.

CONCLUSION

The photochemically generated products from (alkyl-substituted) *N*-acetoacetyl amino acid methyl esters **1a–c** after UV irradiation at 300 nm in acetonitrile were investigated using GC/CI(CH_4)MS. Plausible mechanisms for the major reaction paths are described which involve mainly the photochemistry of the ketone nπ^* triplet. Norrish type I reactions are responsible for the formation of cleavage and coupling products **2–9**.

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