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GAS CHROMATOGRAPHY AND MASS SPECTROMETRY OF DERIVATIVES OF AMINO ACIDS

OXAZOLIN-5-ONES OF SEVERAL ACYL-LEUCINES

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SUMMARY

By the action of dicyclohexylcarbodiimide on fourteen different acyl-leucines in ethyl acetate solution, the corresponding oxazolin-5-ones were formed. The reaction was quantitative and very rapid. The oxazolin-5-ones were separated on a gas chromatograph on OV-7 and EGA columns. The mass spectra of the oxazolin-5-ones showed characteristic fragments that could be used for identification.

INTRODUCTION

Of the numerous derivatives of amino acids that are used in gas chromatographic analysis, acyl-amino acid esters are in most widespread use[1]. However, the carboxyl group of an acyl-amino acid can also form an inner ester linkage with the acyl oxygen atom, resulting in an azlactone or oxazolin-5-one. Oxazolin-5-ones of acyl-amino acids with acyl groups commonly used in amino acid and peptide chemistry, *i.e.*, formyl[2], acetyl[3], trifluoroacetyl[4] and benzoyl[5], have been described in the literature, as well as peptide oxazolin-5-ones[2,6]. The usefulness of such oxazolin-5-ones in gas chromatography has been demonstrated[7,8]. The purpose of the present investigation was to study the formation of oxazolin-5-ones from acyl-leucines with acyl groups of different steric and electronic properties, and to establish whether a mixture of acyl-leucines could be analyzed as the corresponding oxazolin-5-ones on a gas chromatograph and identified on a mass spectrometer.

EXPERIMENTAL

Acyl-leucines with the acyl groups formyl, acetyl, propionyl, *n*-butyryl, isobutyryl, isopentanoyl, *sec.*-pentanoyl, *tert.*-pentanoyl (pivaloyl), lauroyl, crotonyl, cinnamoyl, benzoyl and α-toluyl were obtained by the normal Schotten–Bauman

procedure, *i.e.*, reaction of the acid chlorides with leucine under alkaline conditions. After acidification, isobutyryl- and crotonyl-leucine were isolated as colourless oils, and the others as white crystalline compounds. *tert.*-Butyloxycarbonyl-glycyl-leucine was synthesized as described earlier[8]. The oxazolin-5-ones of acetyl- and pivaloyl-leucine, 2-methyl- and 2-*tert.*-butyl-4-isobutyl-oxazolin-5-one, were synthesized by the action of dicyclohexylcarbodiimide on the acyl-leucines in tetrahydrofuran and purified by distillation under reduced pressure.

Approximately 50 μmole of each of the 14 acyl-leucines were weighed into a 10-ml calibrated flask and dissolved in ethyl acetate. Approximately 725 μmole of dicyclohexylcarbodiimide, *i.e.*, a slight excess over the total amount of acyl-leucines, were added, and the volume was made up to 10 ml with ethyl acetate. A 1-μl aliquot of this solution was chromatographed on a F & M Model 402 gas chromatograph equipped with a flame ionization detector, using a glass column (1.2 m × 3 mm I.D.) packed with 3% OV-7 coated on 80–100 mesh Chromosorb W AW DMCS. Argon was used as the carrier gas at a flow-rate of 35 ml/min. The resulting chromatogram is shown in Fig. 1. Similarly, a mixture of the acyl-leucines with aliphatic acyl groups,

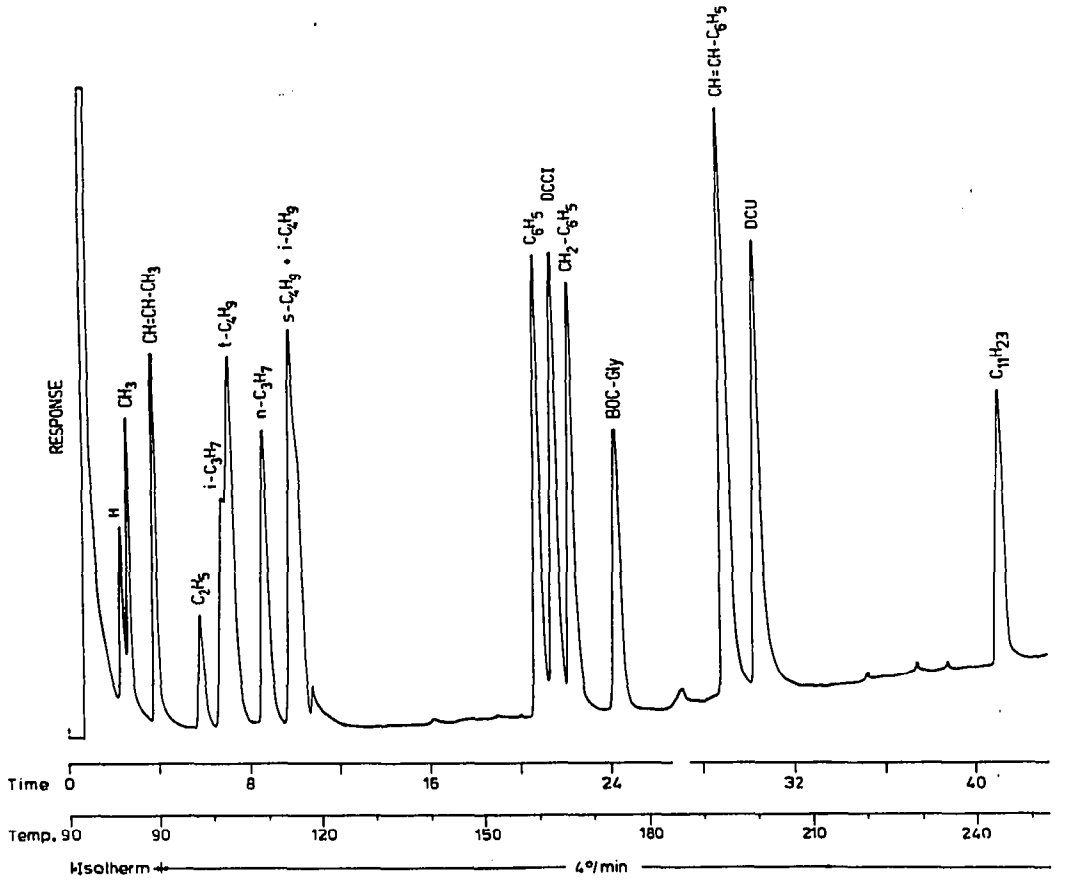


Fig. 1. Chromatogram on OV-7 of a mixture of fourteen 4-isobutyl-oxazolin-5-ones with different substituents on position 2. Abbreviations: DCCI, dicyclohexylcarbodiimine; BOC, *tert.*-butyloxycarbonyl; DCU, dicyclohexylurea.

except lauroyl-leucine, were treated with dicyclohexylcarbodiimide and chromatographed on a column of 0.325% EGA coated on 80–100 mesh Chromosorb G AW. The chromatogram is shown in Fig. 2.

Mass spectra were obtained on a Varian Mat 111 combined gas chromatograph–mass spectrometer, and high-resolution mass spectra on an AEI MS 902 instrument with a DS 30/64/HL data system.

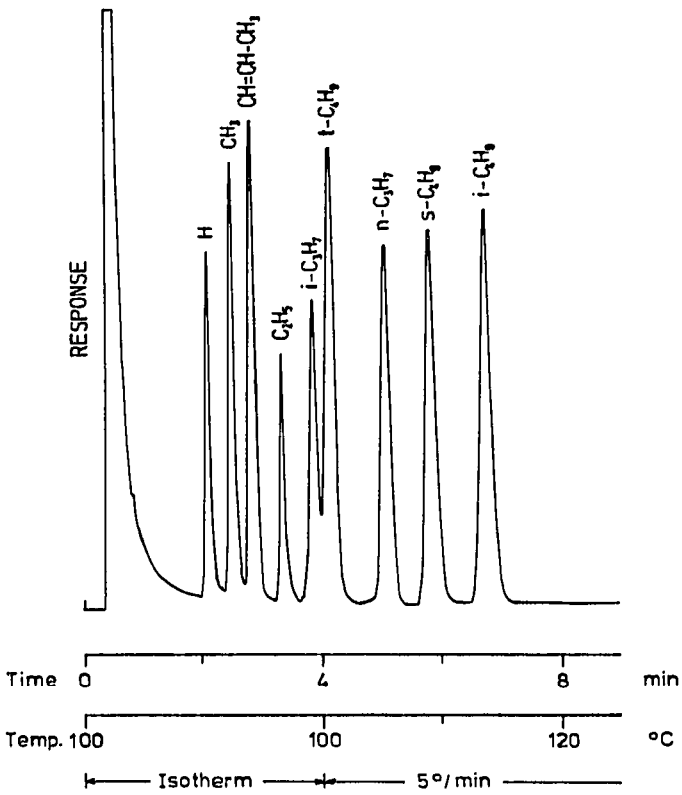
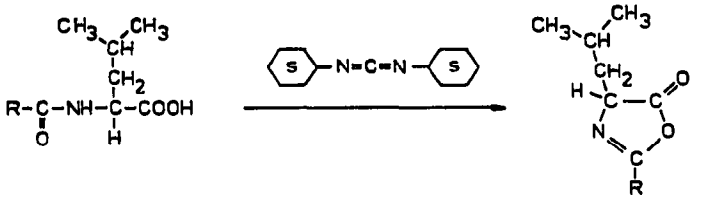


Fig. 2. Chromatogram on EGA of a mixture of nine 4-isobutyl-oxazolin-5-ones with different substituents on position 2.

RESULTS AND DISCUSSION

The facile formation of oxazolin-5-ones from acyl-amino acids has been known since 1908[9]. It is an undesirable side reaction in peptide synthesis because it nearly always leads to unwanted racemisation. Dicyclohexylcarbodiimide is used extensively in peptide synthesis. It strongly activates free carboxyl groups of amino acids or peptides for nucleophilic attack, and with the acyl oxygen atom as the only available nucleophile, the oxazolin-5-one ring is readily formed:



Under the conditions used (room temperature and a 5 m*M* solution of each of the acyl-leucines in ethyl acetate), the ring formation seemed to be completed in a very short time; no significant difference in the chromatograms obtained after 2 min and after several hours could be detected. Likewise, differences in the size of the acyl groups, *i.e.* between hydrogen and *tert.*-butyl, and differences in electronic effects of the acyl groups had no detectable influence on the ring formation under the conditions used.

Several solutions of known concentrations of 2-methyl-4-isobutyl-oxazolin-5-one as well as several solutions of known concentrations of acetyl-leucine plus a slight excess of dicyclohexylcarbodiimide were chromatographed. By comparison of the peak areas, it was found that the oxazolin-5-one-forming reaction was quantitative.

The oxazolin-5-ones had good chromatographic properties and symmetrical peaks were obtained. On the OV-7 column, all of the oxazolin-5-ones were separated, with two exceptions: the isopropyl derivative was eluted as a shoulder in front of the peak of the *tert.*-butyl derivative, and the isobutyl and *sec.*-butyl derivatives were eluted in a single peak. Complete separations of these derivatives were obtained, however, on the EGA column (Fig. 2). The dipeptide oxazolin-5-one was not eluted from the EGA column. On the other hand, the OV-7 phase was found to give a slightly better separation than the more commonly used OV-17.

The retention times increased with increasing molecular weight. The 1-propenyl derivative was more volatile than the saturated *n*-propyl derivative. Among the isomers, increased branching of the side chain increased the volatility. The retention time for the *tert.*-butyl derivative was in fact lower than for the *n*-propyl derivative. The reaction product of dicyclohexylcarbodiimide, dicyclohexylurea (DCU), was also eluted from the column.

Abundant mass spectral fragments that could be used for identification of the oxazolin-5-ones are listed in Table I. The following brief discussion of the mass spectra is supported by the elemental composition of the fragments determined from the high-resolution spectra of 2-methyl- and 2-*tert.*-butyl-4-isobutyl-oxazolin-5-ones.

All compounds had molecular ions; for those with aliphatic 2-substituents, the intensity was very low, while for those with aromatic and unsaturated 2-substituents, the intensity was significantly higher. $(M-CH_3)^+$ and $(M-H_2O)^+$ fragments were also observed for all derivatives, but the intensities were low. The fragment resulting from loss of CO, $(M-28)^+$, was also characteristic; it was of low abundance for all but the *n*-propyl and *sec.*-butyl derivatives, which had 14% and 8% intensity, respectively, of this fragment.

In these two cases, the $(M-28)^+$ ion was probably due to an entirely different fragmentation, namely cleavage between the α- and β-carbon atoms of the 2-substit-

TABLE I

MASS NUMBERS OF THE MOLECULAR IONS AND RELATIVE INTENSITIES OF CHARACTERISTIC FRAGMENTS IN THE MASS SPECTRA OF 2-R-4-ISOBUTYL-OXAZOLIN-5-ONES

R	*m/e of M*	*Relative intensity*									
		M	*M−15*	*M−18*	*M−28*	*M−42*	*M−43*	*M−56*	*M−70*	*M−112*	*M−140*
Hydrogen	141	0.5	4	2	2	16	14	60	100	—	—
Methyl	155	0.1	0.2	0.6	0.2	5	9	22	25	100	—
Ethyl	169	0.8	0.6	1.3	0.5	1.5	12	41	41	100	—
n-Propyl	183	1	1.2	0.8	14	1.5	17	33	41	51	100
Isopropyl	183	1.3	1.3	1.3	0.6	2	12	21	29	35	100
Isobutyl	197	0.8	1.2	0.8	0.8	28	14	18	26	42	77
sec.-Butyl	197	0.7	1	0.5	8	1.3	13	23	13	37	100
tert.-Butyl	197	2	1.5	1.5	0.5	2	16	26	40	38	100
Phenyl	217	6	0.5	2	0.3	1	9	10	10	100	45
Benzyl	231	4	0.5	0.5	0.1	1.5	5	7	10	8	100
1-Propenyl	181	4	2	2	1	2	21	21	27	100	39
Styryl	243	10	1	1	1	3	16	15	8	100	30

uent to furnish the $(M-C_2H_4)^+$ fragment. The formation of this fragmentation was possible only for the oxazolin-5-ones with *n*-propyl and *sec.*-butyl substituents, and could be used to distinguish between the two propyl derivatives, and also to distinguish the *sec.*-butyl from the other two butyl derivatives.

Los of 42 mass units gave abundant fragments, due to loss of C_3H_6 from the isobutyl side chain. The three isomeric butyl derivatives showed a significant difference in this fragment; the isobutyl derivative had an $(M-42)^+$ fragment that was about 15 times more abundant than for the other two, showing that C_3H_6 was released from the isobutyl group in position 2 more easily than from the isobutyl group in position 4.

The $(M-43)^+$ fragment resulted from two different fragmentations: loss of C_3H_7 from the side chain and CH_3CO from the ring. The $(M-C_3H_7)^+$ fragment was the most abundant, and was also more abundant than $(M-C_3H_6)^+$.

The isobutyl substituent in position 4 was cleaved off via a McLafferty rearrangement as the isobutene moiety[10], leaving the enol fragment of the ring, $(M-C_4H_8)^+$, with intense peaks for all derivatives, although the phenyl and benzyl derivatives gave lower intensities than the others.

Fragmentation of the oxazolin-5-one ring occurred through several pathways. Loss of C_4H_6O resulted in the $(M-70)^+$ ion, and may have resulted from cleavage of C_3H_6 from the isobutyl side chain and loss of carbonyl from the ring; both fragmentations were also observed independently. Other fragmentations of both the 4-substituent and the ring gave rise to a group of characteristic fragments: $(M-84)^+$, $(M-85)^+$ and $(M-87)^+$ as well as $(M-98)^+$.

$(M-112)^+$ was a very intense peak for all of the derivatives, and for several it was the base peak (the $(RCO)^+$ fragment). For the methyl and ethyl derivatives, this corresponded to acetyl and propionyl ions, which were the most abundant. For the phenyl and the unsaturated derivatives, the $(RCO)^+$ fragment also gave the largest peak. The high stability of these fragments could be attributed to the conjugation of the carbonyl group with the phenyl ring or the double bond. In the case of the benzyl derivative, the $(M-112)^+$ fragment had only 8% relative intensity. How-

ever, for this compound the $(M-113)^+$ ion was very intense (73%). It is likely that the $(C_6H_5CH_2CO)^+$ fragment in this case has lost a hydrogen atom to form the more stable ketene $(C_6H_5CH{=}C{=}O)^+$.

Loss of hydrogen from the $(RCO)^+$ fragment was significant for the aliphatic derivatives also, except for the *tert.*-butyl derivative, which had no hydrogen atom in the α-position to the carbonyl group.

Cleavage of the 2-substituent gave stable $(M-140)^+$ ions. In the cases when this cleavage resulted in a secondary or tertiary fragment, this was the most abundant. The same occurred for the $(C_6H_5CH_2)^+$ ion, the stability of which was enhanced by a probable rearrangement to the tropylium ion.

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