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Note

Characterization of α -keto acids as quinoxalinols

Structure determination of silyl derivatives suitable for gas chromatography

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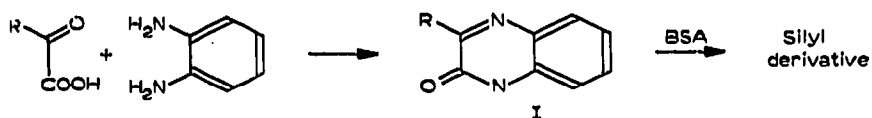
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Recent reports^{1,2} have described the determination of α -keto acids present in biological materials as their silylated quinoxalone derivatives. These are formed by the following sequence and are suitable for use in gas chromatography (GC):



The α -keto acid is made to react with *o*-phenylenediamine to give the quinoxalone³, which is then silylated with bis(trimethylsilyl)acetamide (BSA). It was postulated² that the silylated derivatives formed were those in which the material existed in the amide form (I) and the nitrogen was silylated. During studies on the preparation of halogenated derivatives of α -keto acids suitable for electron capture detection in gas chromatography, we had occasion to study the structure of the silylated derivatives of I by IR and mass spectrometry. In this paper we report our results, which show that the derivatives are entirely in the aromatic form.

APPARATUS

IR spectroscopy was carried out on a Perkin-Elmer 257 spectrophotometer for nujol mulls. Mass spectra were obtained on an LKB 9000 instrument equipped with a gas chromatograph and operating under the following conditions: energy of the ion beam, 70 eV; ion source temperature, 290°; accelerating potential, 3.5 kV; and trap current, 60 μA . GC was carried out with a 2-m glass column packed with 3% OV-17 on Gas-Chrom Q, 80-100 mesh, with a helium flow-rate of 30 ml/min. The column temperature was programmed from 100 to 230° at 10°/min.

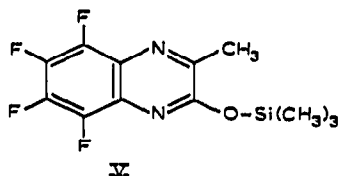
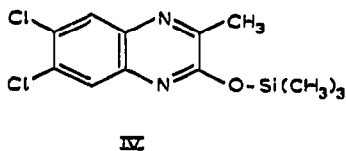
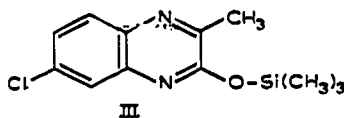
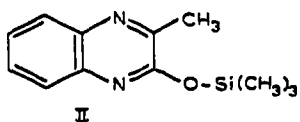
EXPERIMENTAL

The quinoxalones were synthesized by the general procedure of Morrison³ from pyruvic acid and the four phenylenediamines *o*-phenylenediamine, 4-chloro-*o*-phenylenediamine, 4,5-dichloro-*o*-phenylenediamine and 3,4,5,6-tetrafluoro-*o*-phenylenediamine. The melting-points of the quinoxalones obtained were 248°, 252°, 240° and 246°, respectively, all with decomposition.

The silyl derivatives were prepared by the reaction of the quinoxalones with a 1:1 mixture of acetonitrile and bis(trimethylsilyl)trifluoroacetamide (Pierce) at 20° for 30 min in a stoppered vial. The solvents were evaporated in a stream of dry nitrogen immediately before spectroscopic analysis.

RESULTS AND DISCUSSION

Each of the silyl derivatives II, III, IV and V showed a single clean peak on GC analysis and the mass spectrum for each was obtained by combined GC-mass spectrometry.



The mass spectrum of each silylated derivative showed essentially the same fragmentation pattern, and Fig. 1 shows both the mass spectrum and its interpretation for derivative II. Each shows a loss of the $-\text{OSi}(\text{CH}_3)_3$ moiety, indicating that the silyl group is attached to the oxygen atom. This fragmentation could arise through a rearrangement process⁴, but in fact does not, as shown by the IR evidence. Each of the derivatives shows a molecular ion, and a base peak due to the loss of 15 a.m.u., a methyl radical.

In order to obtain good, clear IR spectra, the silyl derivatives were prepared by using a mixture of bis(trimethylsilyl)trifluoroacetamide and acetonitrile, rather than pyridine as solvent, which causes difficulties in evaporation and isolation of the derivative.

The silyl derivatives showed IR spectra consistent with the silyl group being attached to the oxygen. There was no significant absorption in the carbonyl region except for the non-halogenated derivative II, which showed a small absorption at 1670 cm^{-1} (5%), probably due to decomposition of the extremely water-sensitive compound. These results are in contrast to those of Hoffman and co-workers^{1,2}, who reported that their spectral data are in accord with the silyl derivatives having an

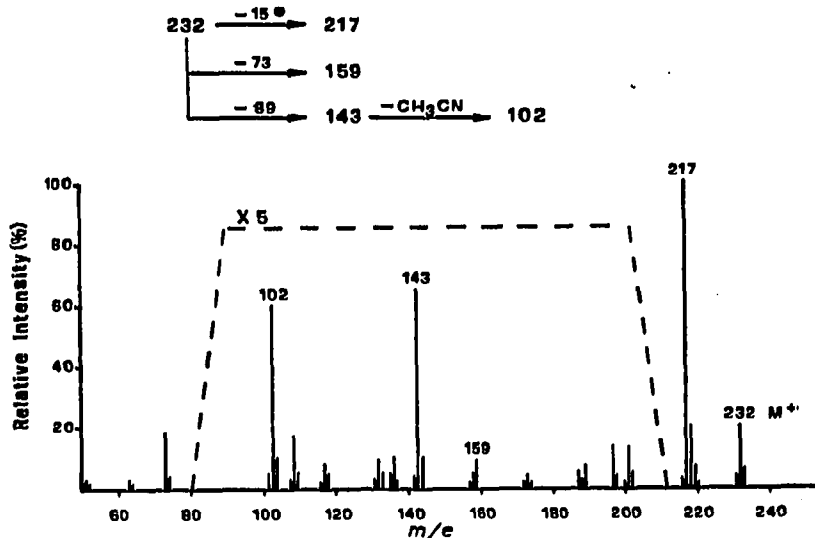
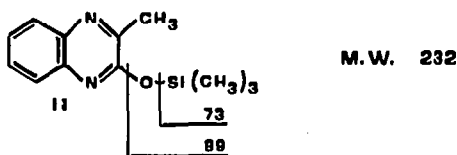


Fig. 1. Mass spectrum and its interpretation for derivative II.

amide structure, but no IR data were given. Characteristic bands present in the IR spectra of derivatives II-V were those at $1415\text{--}1430\text{ cm}^{-1}$ (Si-CH₃) and $850\text{--}860\text{ cm}^{-1}$ (Si-CH₃), with no carbonyl absorption at 1670 cm^{-1} .

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