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BIOCHEMISTRY
DEPARTMENT R & D PLANT PROTECTION
AGROCHEMICALS DIVISION
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Basle, Switzerland

PROJECT REPORT 06 / 79

IDENTIFICATION OF METABOLITES OF CGA 48 988 (RIDOMIL®) IN GRAPEVINE

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Basle, Switzerland, March 8, 1979

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ABSTRACT

Continuing earlier studies on the metabolism of CGA 48 988 in grapevine, the following metabolites were identified by chromatographic and spectroscopic means:

- Two atropisomeric forms of N-(2-methyl-6-hydroxymethylphenyl)-N-(2'-methoxyacetyl)-alanine methylester,
- N-(2,6-dimethyl-3-hydroxyphenyl)-N-(2'-methoxyacetyl)-alanine methylester,
- N-(2,6-dimethylphenyl)-N-(2'-methoxyacetyl)-alanine, and
- N-(2,6-dimethylphenyl)-N-(2'-hydroxyacetyl)-alanine.

These metabolites give evidence for independend degradation pathways which utilize ring methyl oxydation, ring hydroxylation and hydrolysis of the methylester and methylether bonds. All metabolites thus formed were found partially conjugated with sugars.

The content of CGA 48 988 and its metabolites in mature plants was reinvestigated in a new field experiment after six regular applications of the fungicide. Totally 1.4 ppm and 0.9 ppm CGA 48 988 equivalents were found in the whole grapes and in the juice, respectively. The portion of unchanged CGA 48 988 was 0.83 ppm in the grapes and 0.56 ppm in the juice.

1. INTRODUCTION

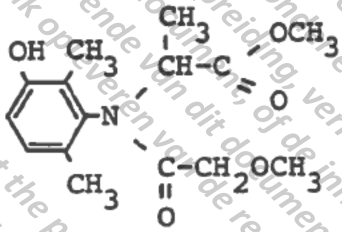
In a previous report (1) the results of a study on the fate of the fungicide CGA 48 988 (RIDOMIL®) in grapevine have been presented. However, the nature of the metabolites found was only tentatively characterized. Therefore, additional efforts were undertaken to elucidate the genuine character of the metabolites and to determine their quantitative significance. The results obtained are reported below.

2. RESULTS AND DISCUSSION

2.1 Identification of metabolites

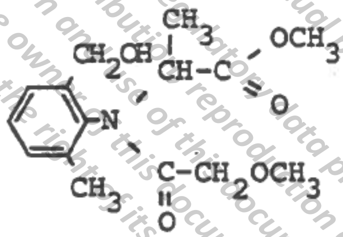
As previously reported, all of the water soluble radioactivity of leaves and grapes (divided in presscake and juice) became methylene chloride soluble after incubation with cellulase. TLC in a two-dimensional system showed, that this radioactivity consisted of the same metabolites (zones I through IV) which were already found in the original methylene chloride phase, indicating that all metabolites were present in their free and conjugated form. Two-dimensional TLC of both phases with solvent system 60/103 A are shown in Figures 1 and 2. These fractions were quantitated and the results are summarized in Table II.

<u>Zone I</u> behaved on TLC in different solvent systems as CGA 100 255. The GLC/MS analysis showed identical retention time and significant m/e peaks (see Figure 3) for the zone I and reference CGA 100 255, thus confirming the identity of this fraction with the meta-hydroxylated parent CGA 48 988:



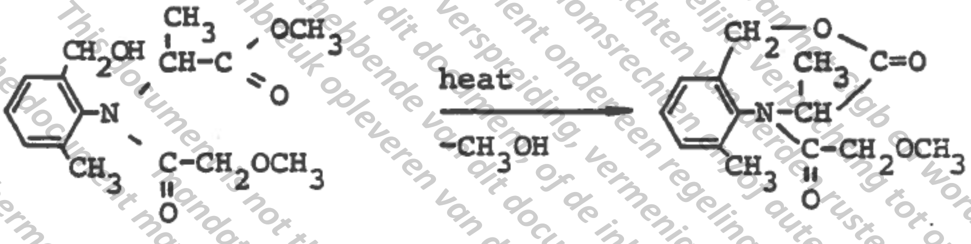
CGA 100 255

<u>Zone II and zone III</u> co-chromatographed on TLC with the two atropisomeric forms of CGA 94 689:



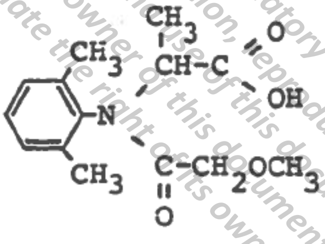
CGA 94 689

The identity of the two metabolites with the synthesized isomers of CGA 94 689 was confirmed by MS (direct probe, Figure 4) and GLC/MS (Figure 5 and 6) of the silylated and underivatized compounds. The M^+ 263 obtained for the underivatized metabolites by GLC/MS can be explained by an intramolecular rearrangement during GLC separation involving cleavage of methanol and formation of a lactone:



M 295 M 263

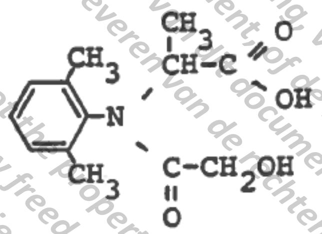
Zone IV consisted of two polar metabolites (ratio 1:1) as demonstrated by TLC in solvent system 103 A. The reference compound CGA 62 826 corresponded to one of them :



CGA 62 826

To confirm the identity of this carboxy metabolite it was converted to the corresponding methyl ester CGA 48 988 and submitted to TLC and GLC. In both systems the methyl derivative behaved identically to the reference CGA 48 988.

Methylation of the second polar compound formed a derivative which co-chromatographed on TLC and GLC with CGA 67 869. Consequently, the genuine structure of this compound must be the acid alcohol derivative:



Based on the structures reported above metabolic pathways for the degradation of CGA 48 988 are proposed in Figure 7. It shows, that the degradation of CGA 48 988 in grapevine proceeds primarily via three independent pathways:

1. oxidation of one of the ring methyl groups
2. ring hydroxylation
3. hydrolysis of the methyl ester and ether bonds

All the metabolites thus formed are efficiently conjugated with sugars.

2.2 Reevaluation of the recovery and distribution of radioactivity

As in the former field experiment (1) a great part of the plant material was lost during a hailstorm shortly before the last treatment, exact establishment of the radioactivity balance was not possible.

Therefore, a new field experiment was performed (for details see 3.2). The spraying calendar and rate of application were slightly changed to comply with the latest recommendations for practical use.

The figures for recovery of radioactivity and final residues in the different plant parts are compiled in Table III.

The results show that less than 3 % of the radioactivity totally applied were present in the plants at maturity. The low recovery was probably due to drift during the application and volatilization of CGA 48 988 and, possibly, some of its degradation products immediately after the treatment.

The content of CGA 48 988 and its metabolites in the leaves and grapes in this experiment was lower than that found earlier in the plants damaged by a hailstorm (1). The difference is large especially in the presscake corroborating the assumption that in the earlier experiment, due to massive leave losses, the grapes were contaminated by direct application during the last treatment.

3. MATERIALS AND METHODS

If not otherwise stated the same materials and methods were used as described in the earlier experiment (1).

3.1 Identification of metabolites
Mass spectrometry

Metabolite zones I through III were identified by electron-impact mass spectrometry using a Finnigan mass spectrometer system, model 4000 (Finnigan Instruments Corp., Sunnyvale, Calif., USA).

3.2 Reevaluation of the distribution of radioactivity

The specific activity of the ^{14}C-CGA 48 988 used was 3.0 µCi/mg.

Two grapevines were sprayed six times until run-off with a spray mixture of ^{14}C-CGA 48 988 formulated as WP 25 (A-5505) at a concentration of 30 g a.i./100 l in water.

The spraying calendar followed is given below:

No.	Date	ml sprayed per plant
1	June 9, 1978	70
2	June 22	100
3	July 6	150
4	July 20	200
5	August 3	300
6	August 18	400

Leaves and ripe grapes were harvested on October 24, 68 days after the last application.

The grapes were shredded in a food cutter and pressed. The grape juice had a density of 1.07 corresponding to 70° Oechsle indicating a moderate sugar content. Leaves and presscake were exhaustively extracted with methanol/water 8:2. The non-extractable radioactivity was determined by combustion of the dry residue to $^{14}CO_2$. After evaporation of the methanol the aqueous residues of the extracts as well as the juice were extracted three times with methylene chloride. The amount of the residual parent compound was determined by co-TLC of the different methylene chloride phases with CGA 48 988.

4. ACKNOWLEDGEMENT

The skilled technical assistance of Miss [redacted] is gratefully acknowledged.

MS analysis was performed by Dr. [redacted], Residue Analysis, Agrochemicals Division, CIBA-GEIGY Limited, Basle, Switzerland.

Non-labelled reference compounds were synthesized by Dr. [redacted] [redacted] CIBA-GEIGY Corporation, Agricultural Division, Greensboro, N.C., USA.

5. REFERENCES

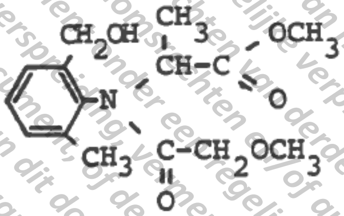
1. [redacted], Metabolism of CGA 48 988 in Grapevine, Project Report 11/78, Biochemistry, Department R & D Plant Protection, Agrochemicals Division, CIBA-GEIGY Limited, Switzerland.

... [redacted] ...

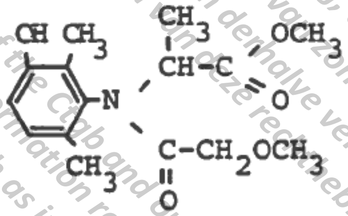
6. TABLES AND FIGURES

Table I Code numbers and structures of reference compounds mentioned

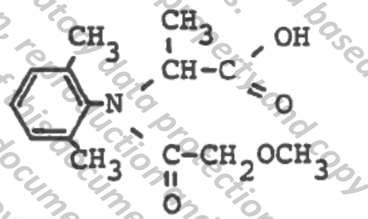
CGA 94 689*



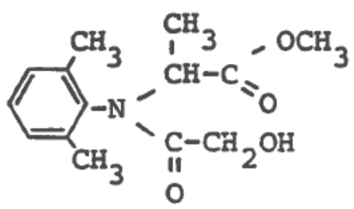
CGA 100 255



CGA 62 826



CGA 67 869



* exists in two atropisomeric forms

Table II Pattern of metabolites in grapevine at harvest time
(in % of the radioactivity found in grape and leaves, respectively)

	CGA 48 988	Zone I		Zone II		Zone III		Zone IV		Non-extractable
		free	con-jugat.	free	con-jugat.	free	con-jugat.	free	con-jugat.	
Grapes										
Juice	7.8	1.5	0.2	1.1	0.2	3.5	2.2	0.9	0.1	-
Presscake	56.3	2.0	0.6	1.0	0.4	6.2	5.8	0.6	0.2	9.4
Total	64.1	3.5	0.8	2.1	0.6	9.7	8.0	1.5	0.3	9.4
Leaves	22.4	8.0	5.0	4.5	2.8	18.7	29.4	3.0	2.0	4.2

Table III Content of radioactivity in grapevine at maturity

		Recovery of radioactivity [%] 1)	Residues [ppm] total 2)	CGA 48 988 3)
Grapes	Juice	0.06	0.9	0.56
	Press-cake	0.12	1.7	0.96
	Total	0.18	1.4	0.83
Leaves		2.38	19.8	2.90

1) Given in % of the radioactivity totally applied

2) Given as CGA 48 988-equivalents

3) Evidenced by co-TLC of the extracts with reference CGA 48 988

Figure 1 : Original CH_2Cl_2 -phase of the leaves

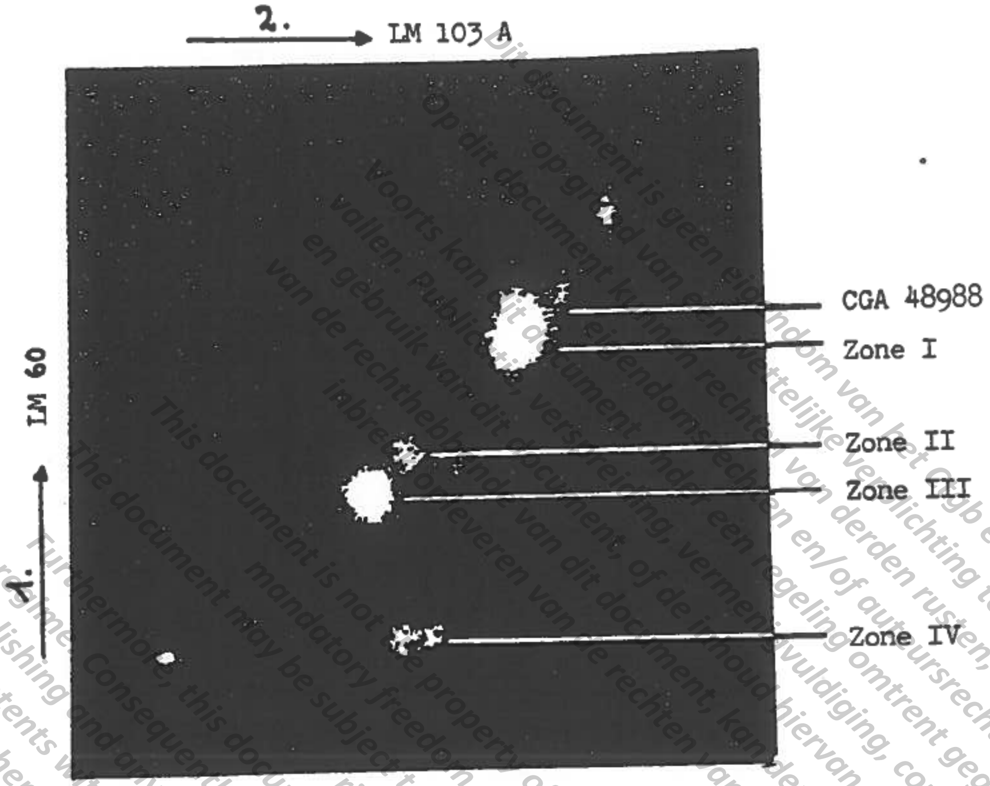
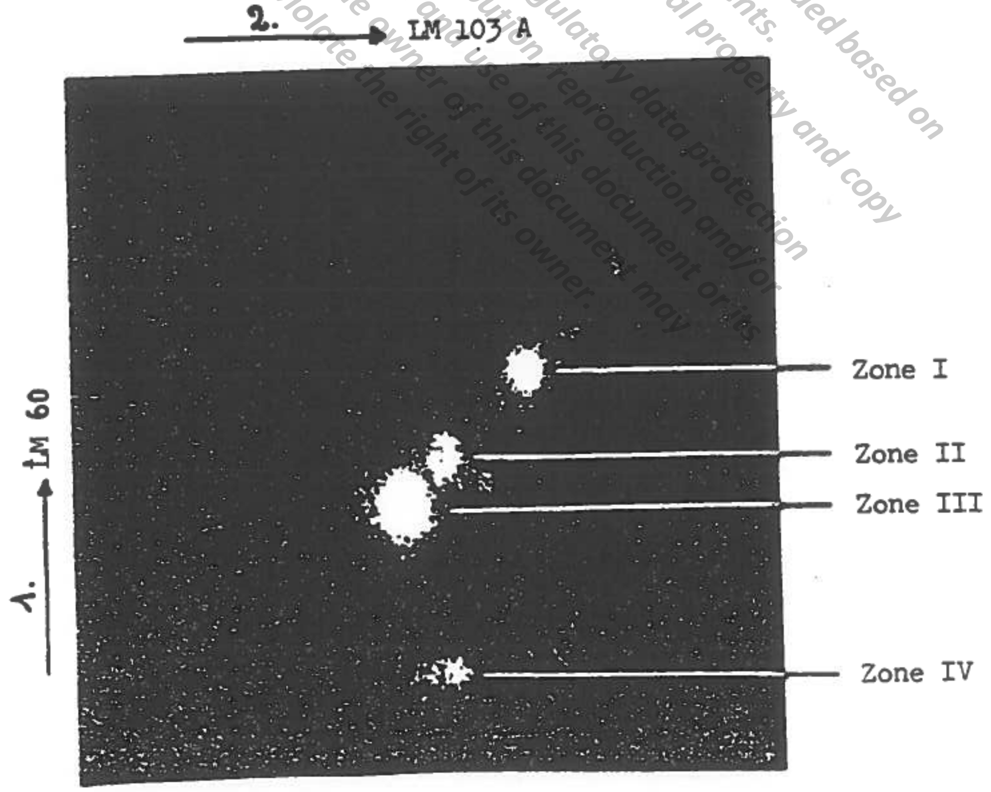


Figure 2 : CH_2Cl_2 -phase of the water soluble radioactivity of the leaves after incubation with cellulase



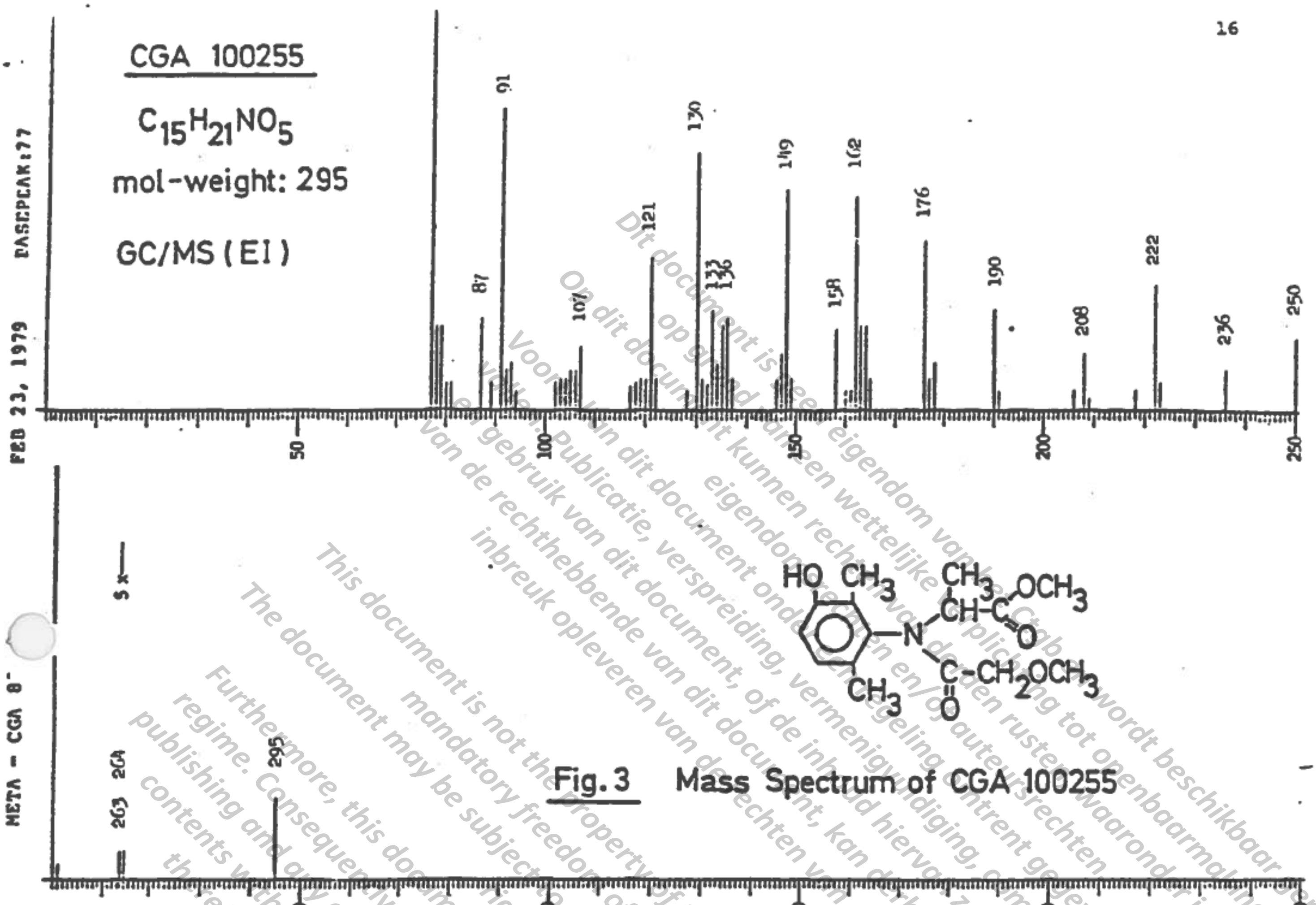


Fig. 3 Mass Spectrum of CGA 100255

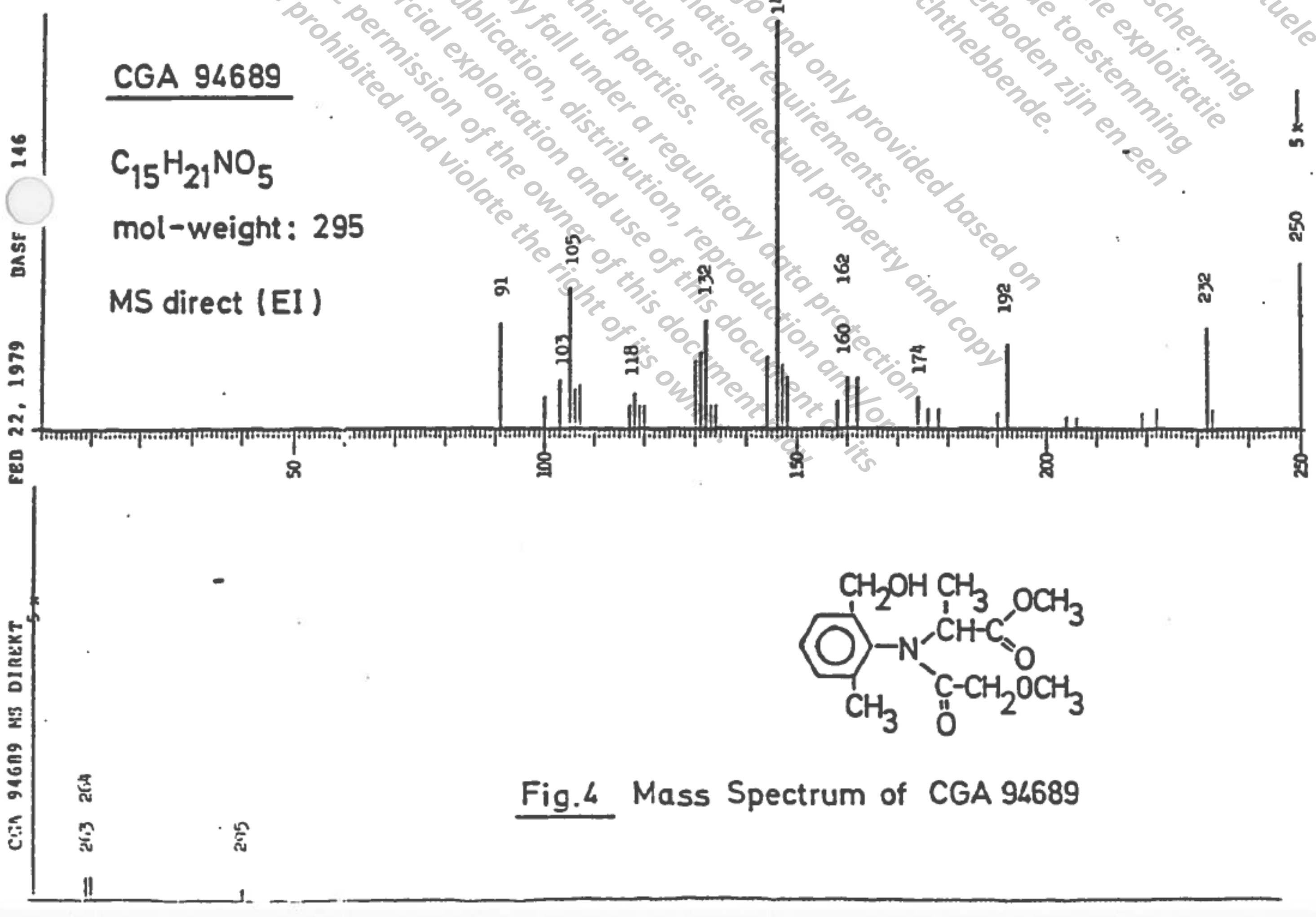


Fig. 4 Mass Spectrum of CGA 94689

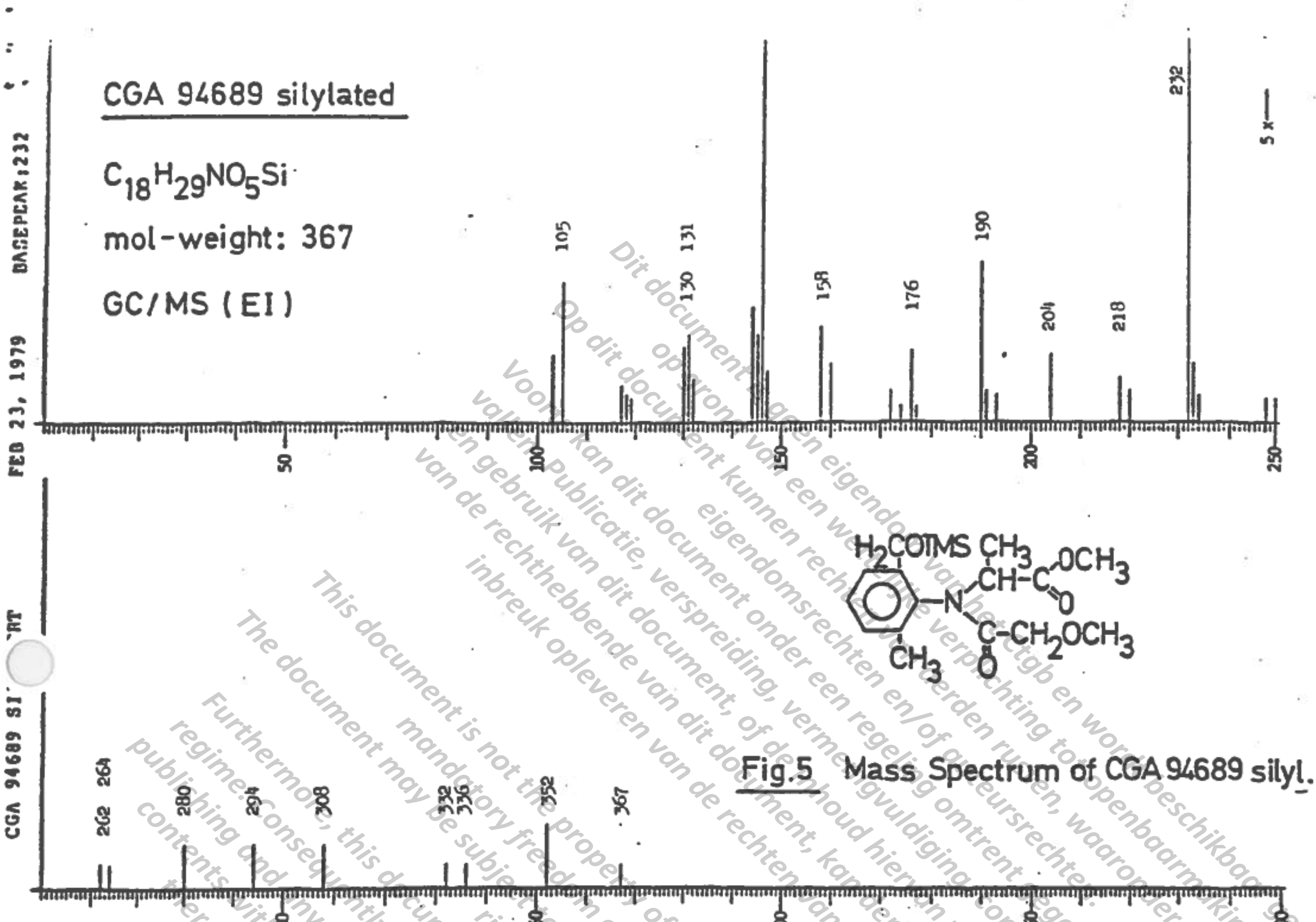


Fig.5 Mass Spectrum of CGA 94689 silyl.

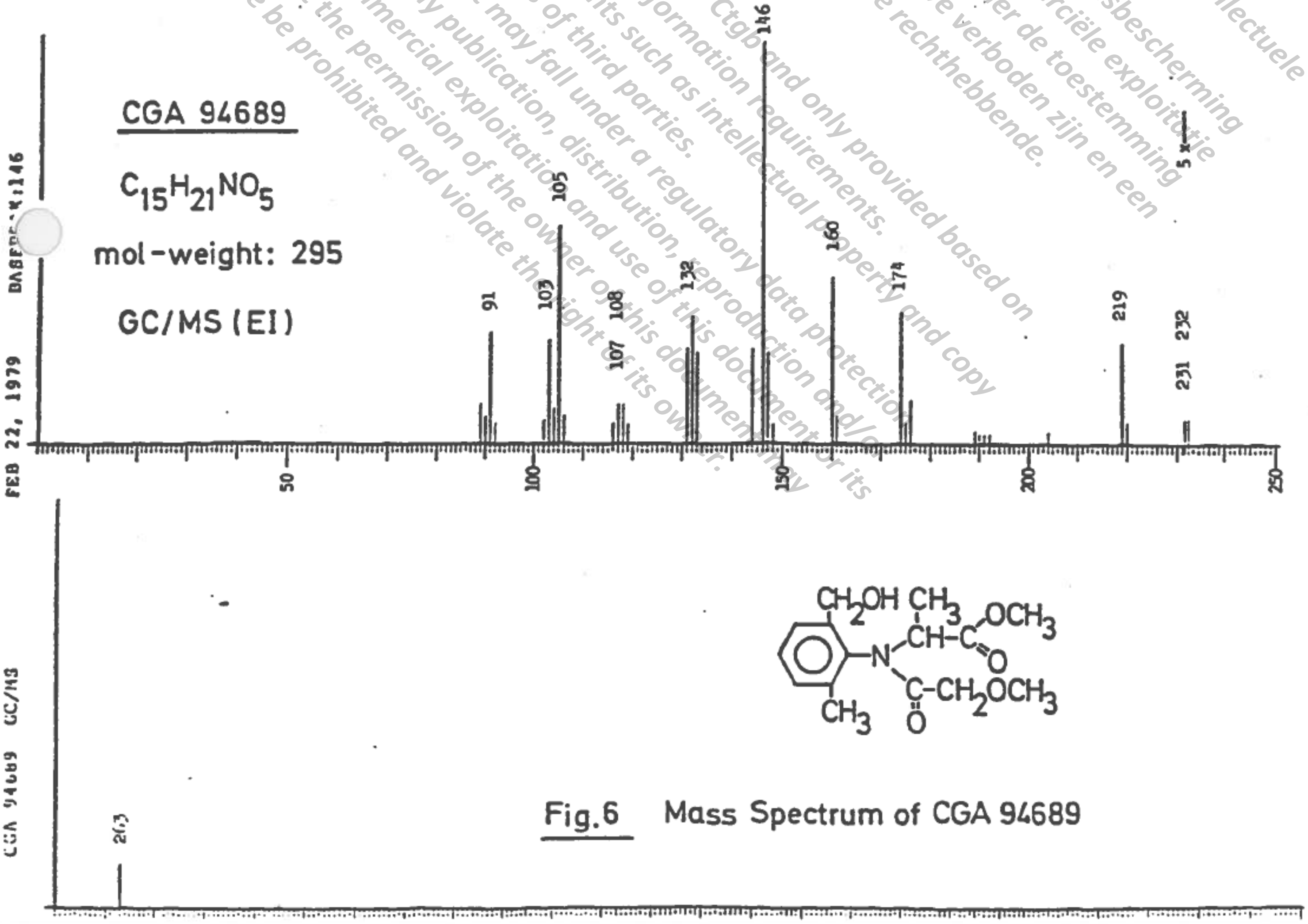
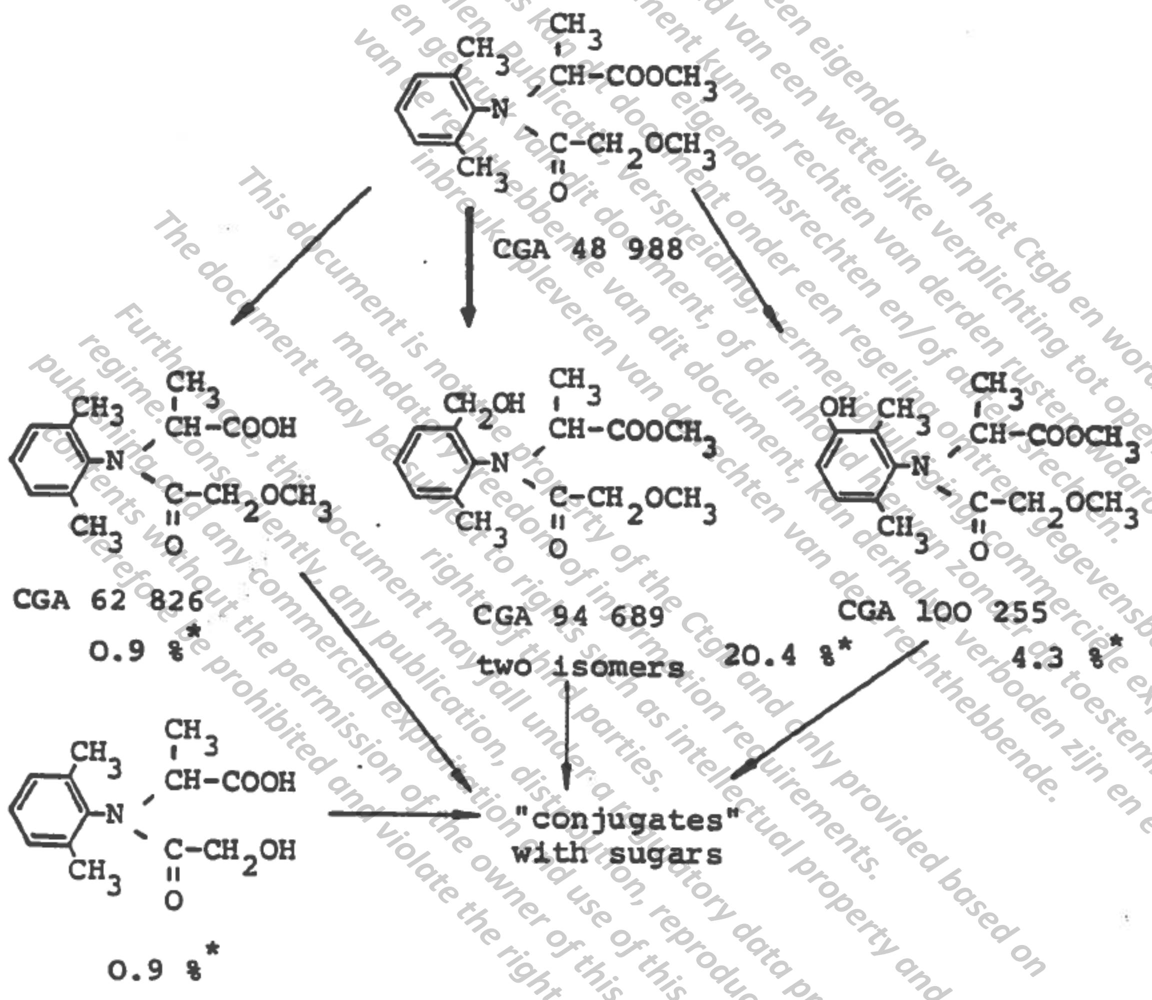


Fig.6 Mass Spectrum of CGA 94689

<u>Figure 7</u> Metabolic pathways proposed for the degradation of CGA 48 988 in grapevine



* percentage of the free and conjugated forms of the metabolites in grapes