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IDENTIFICATION OF THE MAJOR AGLYCONES OF ϕ-^{14}C-CGA-48988 CONJUGATED METABOLITES IN CURED GREENHOUSE GROWN BRIGHT TOBACCO

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ABSTRACT

In the greenhouse, bright tobacco slips were treated at 0.5 lb. ai/acre with ϕ-^{14}C-CGA-48988 and 19 weeks later mature top leaves harvested and cured. These cured leaves contained 93.7 ppm equivalent to ϕ-^{14}C-CGA-48988. This cured tobacco was used to identify four key aglycones to determine the metabolic pathways of CGA-48988. The radioactive aglycones designated II', III', IV' and VI' were each isolated, purified and identified by GLC/Mass spectrometry, GLC/Fourier Transform Infrared spectrometry, and two dimensional TLC. Parent CGA-48988 composed 15% of the total radioactivity. Aglycone II' (for structures see Figure 14) was identified as the meta-phenol of CGA-48988 and composed 14.7% of the radioactivity. Aglycone III', a benzyl alcohol of CGA-48988 which is an optically active atropisomer (A), composed 4.7% of the total ^{14}C; IV', also a benzyl alcohol which is an optical atropisomer (B), composed 10.6% of the ^{14}C; and VI', an acidic alcohol which is demethylated CGA-62826, composed 16% of the total radioactivity.

Individual KOH fusion of aglycone II', VI' and the benzyl alcohol of CGA-48988 (a standard) supported the above identification. As expected the phenolic aglycone II' yielded no ϕ-^{14}C-2,6-dimethylaniline. The acidic alcohol VI' was partially (64%) converted to ϕ-^{14}C-2,6-dimethylaniline. The benzyl alcohol standard gave a characteristic yield of 2,6-dimethylaniline (36%) and o-toluidine (32%). These data are consistent with the assigned structures of II', VI', III' and IV'. These recoveries of 2,6-dimethylaniline support a metabolic pathway consistent with data which showed that only 39% of the total radioactive metabolites in cured tobacco leaves could be converted to 2,6-dimethylaniline and 19% to o-toluidine using KOH fusion.

A metabolic pathway for CGA-48988 in tobacco is proposed. Competitive hydrolysis, oxidation and hydroxylation of CGA-48988 lead to a multiplicity of intermediate metabolites which are acids, alcohols, and phenols and which are rapidly converted to multiple sugar conjugates.

The metabolic oxidation of the ring methyl group and the hydroxylation of the benzene ring to form phenols result in metabolites which do not give complete conversion to the common moieties, 2,6-dimethylaniline and o-toluidine, using a KOH fusion. In a top cured leaf the free and conjugate phenols may amount to 15% of the total residue.

INTRODUCTION

The experimental compound CGA-48988, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester*, is a fungicide proposed for control of Black Shank on tobacco. Buckets of soil, each containing one bright tobacco slip, were treated with [U-ring-^{14}C]-N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester** at a rate of 0.5 lb. a.i./A using a transplant water technique. Burley and bright tobacco treated with CGA-48988 (1) showed the same qualitative and quantitative pattern of uptake and metabolism. Bright top leaves harvested 19 weeks after transplanting and then cured were used for identifying four major aglycones of polar metabolites. These major aglycones are designated II', III', IV' and VI'. Figure 3 shows their TLC characteristics. In this 2D-TLC, zone I' is prominent, but for 12-week cured leaves it was not (9). The objectives of this work were to identify four major key aglycones which can be released by enzymes from polar metabolites from both 12- and 19-week cured tobacco and then to propose a metabolic pathway for CGA-48988 in tobacco.

EXPERIMENTAL

Treatment and Radioactive Dose: Coker 319 bright tobacco was treated by a transplant water procedure at 0.50 lb. a.i./A with ϕ-^{14}C-CGA-48988. Details regarding planting, treatment, sampling and curing of the tobacco are in ABR-78036 (1) and Biological Report 78003 (2). Tobacco leaves taken at 19 weeks after treatment and then cured provided the polar metabolites.

Sample Preparation and Analysis: These cured tobacco leaves were homogenized with dry ice in a Wiley Mill (3). Samples of 100-200 mg were combusted in a Harvey oxidizer (4).

Radioactivity Measurements: Radioassays were done in a Beckman LS-255 or Mark III liquid scintillation counter. Efficiencies were obtained by external standardization. Limits of detection and quantitation were determined in accordance with AG-276 (5).

*Chemical names and structures are given in Figure 1.
**Hereafter referred to as ϕ-^{14}C-CGA-48988

<u>Fractionation Scheme:</u> The flow diagram in Figure 2 outlines the steps used for isolating aglycones of ϕ-^{14}C-CGA-48988 metabolites. Forty grams of cured leaves were extracted with 200 ml of methanol and water - 80/20 (v/v). The extract was filtered, concentrated to 100 ml, and then partitioned against 150 ml hexane to give organic solubles ORG 1 and an aqueous phase AQ1. The AQ1 was treated with cellulase (30 mg/ml - Sigma Chemical Co., St. Louis, MO) in acetate buffer at pH 4.6 and at 37°C for 48 hours. The pH was adjusted to 1 with 6 N HCl and partitioned against ethyl acetate four times. The ethyl acetate fraction was concentrated and dissolved in methanol (20 ml). Two hundred ml of water was then added, the pH adjusted to neutral and the sample placed on a 5 cm x 40 cm column of Sephadex A-25 using water. Aglycones II', III', and IV' were eluted with water while V', Va', Vb', and VI' were eluted with 1N KBr.

<u>Isolation and Purification of Aglycones II', III', IV' (Figure 2A)</u>

Aglycones II', III', and IV' were partitioned into glass distilled ethyl acetate, concentrated and separated using preparative thin layer chromatography (Redi Plates-Silica GF 250 microns-Fisher Co., Pittsburgh, PA). The plates were multiple developed three times in heptane:ethyl acetate (50/50-v/v). Each zone was eluted with ethyl acetate and rechromatographed on silica plates which had been washed with glass distilled methanol. The zones II', III', and IV' were eluted separately with ethyl acetate. Each concentrate was applied to an HPLC column (0.4 cm x 25 cm PAC - Whatman Analytical) and eluted isocratically with heptane and 50:50 ethanol:heptane. The Spectraphysics 3500B HPLC used was equipped with a Berthold Radioactive (BF5025) flow cell.

<u>Isolation and Purification of Aglycone VI' (Figure 2B)</u>

The aglycone fraction eluted from the A-25 column with 1N KBr (V', V'a, V'b, VI') was made acidic (pH = 1), partitioned into ethyl acetate, and concentrated. Methanol (3 ml) was added, then 34 ml of Tris-(hydroxy-amino)-methane Buffer (THAM 0.01 m - pH 7.5). This mixture was placed on a 1.5 x 25 cm DE 23 column and eluted with 100 ml of 0.01 M THAM, then with a gradient from 0.01 M to 0.1 M THAM (pH 7.5). Fractions containing radioactivity were pooled, the pH adjusted to 1 with 3N HCl and partitioned against ethyl acetate. The ethyl acetate extract was concentrated, streaked on TLC

plates using methanol washed plates and chromatographed using methanol/chloroform (50/50 - v/v). The lower zone (VI') was scraped from the plate and eluted with glass distilled methanol. The eluate was concentrated and 50 ml of 0.01N HCl added. This aglycone was partitioned into ethyl acetate, concentrated and stored in a refrigerator for 4 weeks. GLC/MS was run on this sample.

Gas Chromatography, RAM Analysis and Mass Spectrometry of II', III', IV', VI'

Gas chromatography of purified aglycones was run using a Hewlett-Packard 5730A gas chromatograph equipped with a 6' 10% DC-200 (Applied Science Laboratories, State College, PA) column and a flame ionization detector. Monitoring of the radioactive peaks was carried out with a Hewlett-Packard 5700 GC equipped with a Searle (Nuclear Chicago) RAM. Retention times were compared to an internal ϕ-^{14}C-CGA-48988 standard (Table II).

Mass spectrometry was done using both a Finnigan model 4000 equipped with an INCOS data system (2100) and a LKB model 2090 equipped with a PDP1104 computer and a LKB preprocessor and MS Program 2031. Both systems were equipped with a 6'-10% DC 200 column at 200°C with an injection port temperature of 250°C. The operating conditions for the Finnigan were: Carrier gas - Helium (30 ml/min.), accelerating voltage 8000, ionizing energy 70 ev, emission current of filament 350 microamps. The source was at 200°C. The conditions for the LKB 2091 were: 30 ml/min. helium gas (GC), accelerating voltage 3500, ionizing energy (E.I.) 70 ev, (C.I.) 150 ev.

Gas Chromatography/Fourier Transform Infrared Spectrometry of Aglycone VI'

Five micrograms of purified VI' were subjected to GLC/FTIR using a Nicolet 7199 Fourier Transform infrared spectrometer equipped with a Nicolet 1180 data system and a Varian 3700 GC. A 10% DC-200 column was used*.

Two Dimensional Thin Layer Chromatography of CGA-48988 Aglycones from Tobacco:

Two dimensional thin layer chromatography was performed on Silica Gel GF Redi TLC plates - 250 microns - 20 x 20 cm (Fisher Scientific, Pittsburgh, PA). The first solvent system was ethyl acetate (saturated) and the second was ethyl acetate/acetic acid (90/10 - v/v - saturated). Visualization of radio-

*Work done at Research Triangle Institute.

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activity was by Spark Chamber (Birchover Corporation) or by exposure to X-ray film (Kodak no screen NS-2T) for one week or more. Radioactivity in a zone was quantitated by scraping it into a scintillation vial, adding 2 ml of methanol and then 15 ml of aquasol and counting.

KOH Fusion of ^{14}C-Aglycones from Tobacco

KOH fusion of ^{14}C aglycones and selected standards was carried out according to ABR-78047 (6).

Synthesis of Aglycone Standards

N-[2-(hydroxymethyl)-6-methylphenyl]-N-(methoxyacetyl)-alanine methyl ester (standard 2, Figure 1) and N-(3-hydroxy-2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester (standard 3, Figure 1) were prepared by Mr. George Boka at Greensboro. CGA-62826 was prepared by Dr. N. Venkateswaran. CGA-68125 was from CIBA-GEIGY, Basel, Switzerland. N-(2,6-Dimethylphenyl)-N-(hydroxyacetyl)-alanine (standard 6) was prepared as follows: 20 mg of CGA-68125 was dissolved in 1 ml 1N NaOH and then concentrated on a rotovap at 28°C. One ml of methanol (glass distilled) and 20 ml of water were added. The pH was adjusted to 5 with 0.1N HCl and the mixture partitioned with 20 ml ethyl acetate (glass distilled). The ethyl acetate fraction was concentrated to dryness and 0.8 ml of methanol plus 0.1 ml water added. NMR and C.I. mass spectroscopy were consistent with the acid-alcohol (standard 6, Figure 1).

RESULTS AND DISCUSSION

Previous characterization of ^{14}C-metabolites, which used cured tobacco prepared from lower Bright leaves harvested 12 weeks after slip transplanting, showed that the polar metabolites could be converted to nine aglycones, 6 of which corresponded to 6 unconjugated metabolites (9). Four of these 9 aglycones are key substances in the metabolism of CGA-48988. Three of these four appear to be neutrals, perhaps alcohols, and one is definitely acidic. Analyses of cured top leaves (19-week) showed a higher percentage of these aglycones than did cured lower leaves (12-week). In the tobacco industry top and middle leaves of Bright tobacco are of greater importance than lower leaves, consequently, cured radioactive top leaves were selected to identify these four key aglycones.

These leaves, containing 93.7 ppm equivalent to ϕ-^{14}C-CGA-48988, were extracted with methanol-water releasing 99% of the radioactivity. Eighty percent was aqueous soluble and 15% was organic soluble (Figure 2A). All of the leaf radioactivity (15%) in the organic fraction was characterized by 2D-TLC as CGA-48988. Figure 3 and Table I show the results of two dimensional TLC of a mixture of free ^{14}C-metabolites and ^{14}C-aglycones from a cellulase digestion of the polar aqueous fraction AQ1. Aglycones II', III', IV', and VI' were found to be key substances in the metabolic pathway of ϕ-^{14}C-CGA-48988 in tobacco. Aglycone I' showed an increase in percent of the total radioactivity when compared to the percentage in 12-week cured tobacco (9). The identification of only II', III', IV' and VI', which were major in 12 and 19 week tobacco, was carried out in this investigation.

The method of fractionation and purification of II', III', IV' and VI' aglycones of polar metabolites from the aqueous fraction is shown in Figure 2. After cellulase digestion of the aqueous soluble radioactivity (AQ1), the ^{14}C-aglycones were released and then partitioned into ethyl acetate (Figure 2). Major aglycones II', III', IV' and VI' were separated by 2D-TLC and make up, respectively, 14.7%, 4.7%, 10.6% and 16.0% of the total ^{14}C in the cured leaf (Table I). Another ten unknown aglycones and/or free metabolites collectively made up (26%) of the radioactivity. The amounts of these aglycones in the top leaves are higher than those shown (9) for lower leaves (ABR-78044, Figure 4, Table I). With time these four aglycones constitute a higher percentage of the radioactivity in treated tobacco.

<u>Identification of Aglycone II'</u>

Aglycone II' was isolated and purified as shown in Figure 2. The chemical ionization mass spectrum obtained using gas chromatography (Table II shows retention time) showed a quasi molecular ion at 296 amu (M+1). Electron impact mass spectral analysis showed a fragmentation pattern (see Figures 4 and 5 and Reference 7) similar to that obtained for N-(3-hydroxy-2,6-dimethylphenyl)-N-(methylacetyl)-alanine methyl ester, the m-phenol CGA-48988 (standard 3, Figure 1) shown below:

OH CH_3 CH_3
CH-$COOCH_3$
N
$COCH_2OCH_3$
CH_3

This standard m-hydroxy CGA-48988 also cochromatographed with isolate II' using 2D-TLC.

Identification of Aglycones III' and IV'

Aglycones III' and IV' were purified and identified indirectly using gas liquid chromatography - mass spectral analysis. The GLC/MS chemical ionization (C.I.) mass spectra indicated that III' and IV' had a molecular weight of 263. The only reasonable structure for such a molecular weight is the lactone:

This lactone was formed by heat on the GLC column from the following aglycone benzyl alcohol.

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When the standard benzyl alcohol was prepared, the GLC/MS (C.I.) of this compound had a quasi molecular ion at 264. By GLC/MS electron impact (E.I.) mass spectrometry, it had the exact same fragmentation pattern as did aglycone III' (Figure 6 and 7). Using the solid probe inlet and chemical ionization mass spectrometry, the benzyl alcohol standard was shown to have a molecular weight of 295 (Figure 8) because masses were detected at M + 1 and M + 29, i.e., 296 and 324.

Aglycone IV' was found to have the same GLC/MS electron impact mass spectrum and chemical ionization mass spectrum (Figure 9) as did III' and the benzyl alcohol standard, indicating that III' and IV' are isomers of each other. Proton NMR studies (8) showed that synthesized isomers A and B of the benzyl alcohol were rotomers (atropisomers) and that III' and IV' were convertible to a lactone (MW 263) upon heating. Using 2D-TLC, it was possible to show (Figure 10) that isomer A corresponded to III' and isomer B to IV'. It was not possible to determine if these ^{14}C-isomers were formed during their isolation and purification or whether they were released as such by the cellulase from different conjugates.

Identification of Aglycone VI'

Acidic aglycone VI' was purified using ion exchange chromatography and preparative TLC (Figure 2B). Upon storage, or when put on a GLC column, VI' partially converted to a neutral material (VI' C1), resulting in a mixture of VI' C1 and the original acid aglycone VI' C2. GLC/MS showed that the non-polar material was the lactone CGA-68125 (see Figures 11A, 11B, 11C, and 12).

GLC of the mixture followed by Fourier Transform IR (FTIR) was used (Figure 13) to confirm that VI' C1 was CGA-68125. These IR spectra show definitive likeness between VI' C1 and CGA-68125 in that a carbonyl (1790 cm^{-1}) group is present in a six membered lactone and a carbonyl group (1715 cm^{-1}) is present in a six membered lactam for both the unknown and standard.

Using the knowledge that VI' C1 was a lactone formed from VI' C2, it was possible to propose a structure for VI' C2:

CH_3 CH_3 CH-COOH N $COCH_2OH$ CH_3
VI'C2

Δ
storage

CH_3 CH_3 CH-C=O N O C-CH_2 O CH_3
VI'C1

Organic synthesis of the authentic acid alcohol (VI' C2) showed that it was indeed converted to CGA-68125 during storage or with heating. If 2D-TLC was run immediately after preparation, the standard cochromatographed with VI' C2. Thus, aglycone VI' C2 is stabilized in vivo by linkage to a sugar as a conjugate. Hydrolysis by cellulase and then storage leads eventually to the artifact CGA-68125 (VI' C1).

KOH Fusion of Aglycones II', III' and VI'

Previous work with 19 week top leaves (6) has shown that 58% of the total cured leaf radioactivity could be related to two common moieties: 2,6-dimethylaniline (2,6-DMA) (39%) and o-toluidine (19%). The ^{14}C-2,6-DMA and o-toluidine are chemical hydrolysis products of ^{14}C-metabolites of tobacco and have never been shown to be free intermediary metabolites in tobacco. Table III shows the results of KOH fusion on CGA-48988 and several of its isolated aglycones. KOH fusion on CGA-48988 shows complete conversion to 2,6-DMA. The phenol (II') shows no conversion to 2,6-DMA, as expected for a phenol. The benzyl alcohol standard partially converts to 2,6-DMA (36%) and to o-toluidine (32%). VI' C1 (CGA-68125) also is partially converted (64%) to ϕ-^{14}C-2,6-dimethylaniline, as would be expected from previous studies (6).

These data support the assigned structures of II', III', IV' and VI' and the presence of these aglycones indicates that only partial recovery of the radioactivity as ^{14}C-2,6-DMA in a hydrolyzed tobacco leaf extract will be achieved by KOH fusion. Since these aglycones exist mostly in conjugation with sugars, and since KOH fusion is not likely to hydrolyze these conjugates, KOH fusion of unhydrolyzed leaf extracts would be expected to give very low recoveries of ϕ-^{14}C-2,6-dimethylaniline.

Metabolic Pathways of CGA-48988

Figure 14 shows the proposed metabolic pathway of CGA-48988 in tobacco. Metabolic attack appears to occur at four locations on CGA-48988:

(1) Oxidation of the aromatic methyl group: This appears to be an oxidation on only one of the methyl groups. Steric hindrance, which can be visualized by building "space filled" models, probably prevents oxidation of the second methyl group.

(2) Oxidation at the meta position of the aromatic ring to form a phenol: Oxidation at the meta position was unexpected and the reason for this nucleophilic attack at the meta position and not the para is not clear at this time.

(3) Hydrolysis of the methyl ester: Hydrolysis of the methyl ester results in a carboxylic acid on the side chain of CGA-48988.

(4) Hydrolysis of the methyl ether: These reactions lead to intermediate metabolites which are alcohols, alcohol-acids or phenols (Figure 14). Many of these intermediate oxidized or hydrolyzed metabolites are shown in Figure 14, but have been characterized in previous studies (9). The alcohols, alcohol-acids and phenols become rapidly conjugated to sugar monosaccharides and possibly disaccharides (9).

Such multiple pathways of oxidation, hydrolysis and conjugation explain the multiplicity of metabolites of CGA-48988 found in cured tobacco leaves. Because of phenolic metabolites, free or conjugated, complete conversion of the residue in top cured leaves will not occur using KOH fusion. In such leaves these metabolites may amount to at least 15% of the total residue.

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TABLE I: QUANTITATION OF CGA-48988 AND ITS AGLYCONES OF ϕ-^{14}C-CGA-48988 METABOLITES IN CURED TOBACCO LEAVES (TOP - 93.7 PPM)

^{14}C-Aglycone or Free Metabolite	Structure	% of Total ^{14}C in Leaf**
CGA-48988	CH_3, CH_3 (ring); N; CH_3–CH-COOCH$_3$; $COCH_2OCH_3$	15.0
I'a	Unknown	1.0
I'	Unknown	8.8
II'	OH, CH_3, CH_3 (ring); N; CH_3–CH-COOCH$_3$; $COCH_2OCH_3$	14.7
II'a	Unknown	1.5
III' A*	CH_2OH, CH_3 (ring); N; CH_3–CH-COOCH$_3$; $COCH_2OCH_3$	4.7

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TABLE I: QUANTITATION OF CGA-48988 AND ITS AGLYCONES OF ϕ-^{14}C-CGA-48988 METABOLITES IN CURED TOBACCO LEAVES (TOP 93.7 PPM) (Continued)

^{14}C-Aglycone or Free Metabolite	Structure	% of Total ^{14}C in Leaf**
III'a	Unknown	1.5
IV' B*	CH$_2$OH; CH$_3$; CH-COOCH$_3$; N; COCH$_2$OCH$_3$; CH$_3$	10.6
V'	Unknown	4.2
V'a	Unknown	2.2
V'b	Unknown	4.1
VI'	CH$_3$; CH$_3$; CH-COOH; N; COCH$_2$OH; CH$_3$	16.0

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TABLE I: QUANTITATION OF CGA-48988 AND ITS AGLYCONES OF φ-^{14}C-CGA-48988 METABOLITES IN CURED TOBACCO LEAVES (TOP 93.7 PPM) (Continued)

^{14}C-Aglycone or Free Metabolite	Structure	% of Total ^{14}C in Leaf**
VII	Unknown	0.8
VIII'	Unknown	1.1
IX'	Unknown	0.4
Total		86.6

*Atropisomers A and B

**The % of total ^{14}C in leaf is a sum of the components occurring as both free intermediary metabolites and as aglycones released by cellulase.

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TABLE II: RETENTION TIMES ON 10% DC-200 FOR CGA-48988 AND AGLYCONES

Compound	Retention Time Min. at 210°C
CGA-48988	5.0
Aglycone II'	11.5
Aglycone III'	9.1
Aglycone IV'	9.1
Benzyl alcohol standard	
Atropisomer A	9.1
Atropisomer B	9.1
Aglycone VI' Cl	4.0
CGA-68125	4.0

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TABLE III: KOH FUSION OF ^{14}C-AGLYCONES OF CGA-48988.

Component Fused	% of Total ^{14}C in Sample Recovered as: 2,6-DMA*	o-toluidine
Cured tobacco leaf (hydrolyzed extract)	55 (40)***	20 (16)
ϕ-^{14}C-CGA-48988	99	0
ϕ-^{14}C-Aglycone II'	0	0
p-Phenol of CGA-48988**	3	0
Benzyl alcohol of CGA-48988 (standard)	36	32
^{14}C-Aglycone VI'Cl (CGA-68125)	64	0

*2,6-DMA = 2,6-dimethylaniline

**nonradioactive material used.

***numbers in parenthesis are percent of total ^{14}C in the cured leaf.

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No.	Structure	Name
1.		CGA-48988 N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester
2. (A)		Benzyl alcohol of CGA-48988 (Atropisomers A and B) CGA-94689 N-[2-(hydroxymethyl)-6-methylphenyl]-N-(methoxy-acetyl)-alanine methyl ester
(B)		
3.		m-phenol of CGA-48988 N-(3-hydroxy-2,6-dimethyl-phenyl)-N-(methoxyacetyl)-alanine methyl ester
4.		CGA-68125

Figure 1: CHEMICAL NAMES AND STRUCTURES

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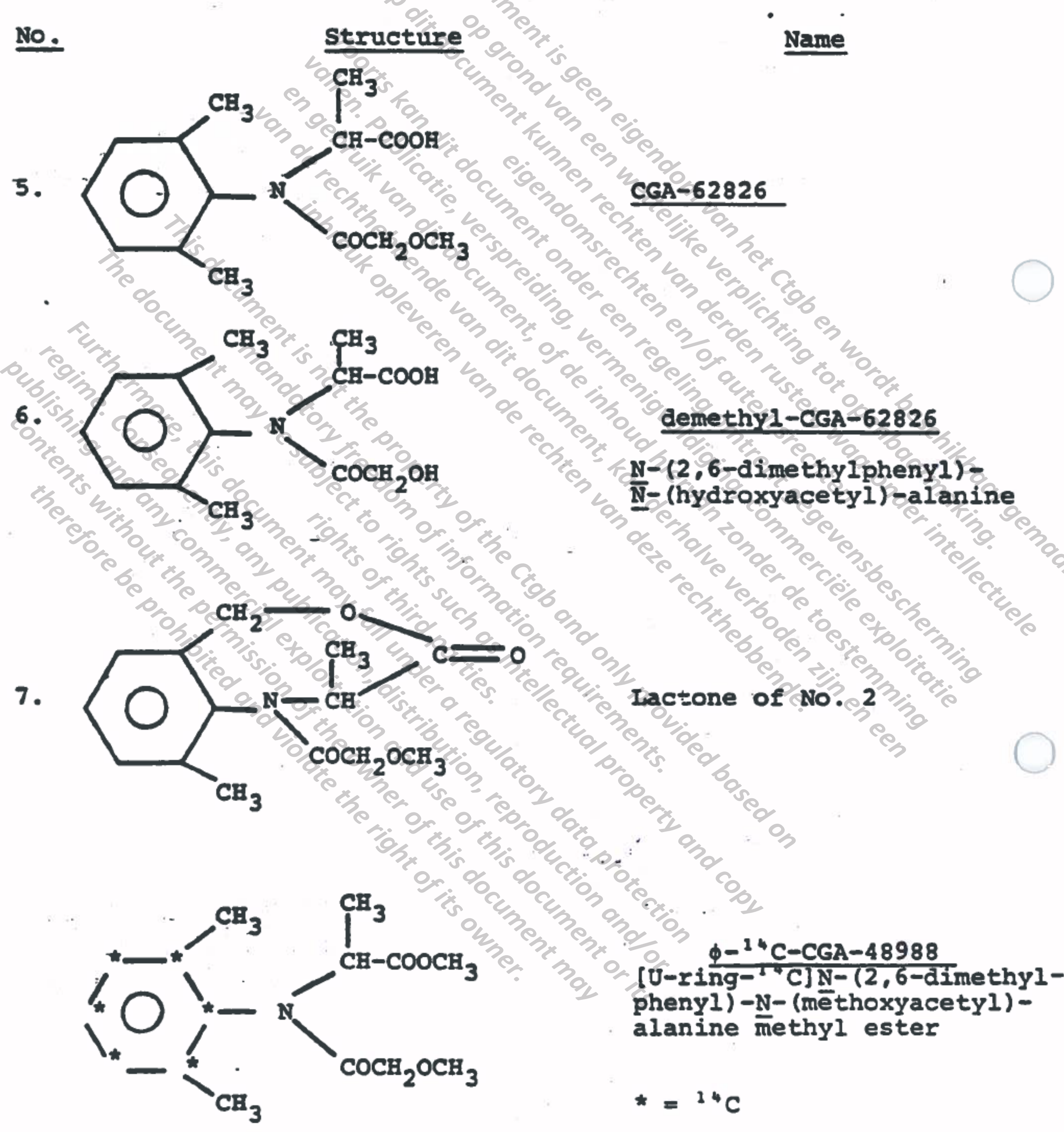

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Figure 1: CHEMICAL NAMES AND STRUCTURES (Cont'd.)

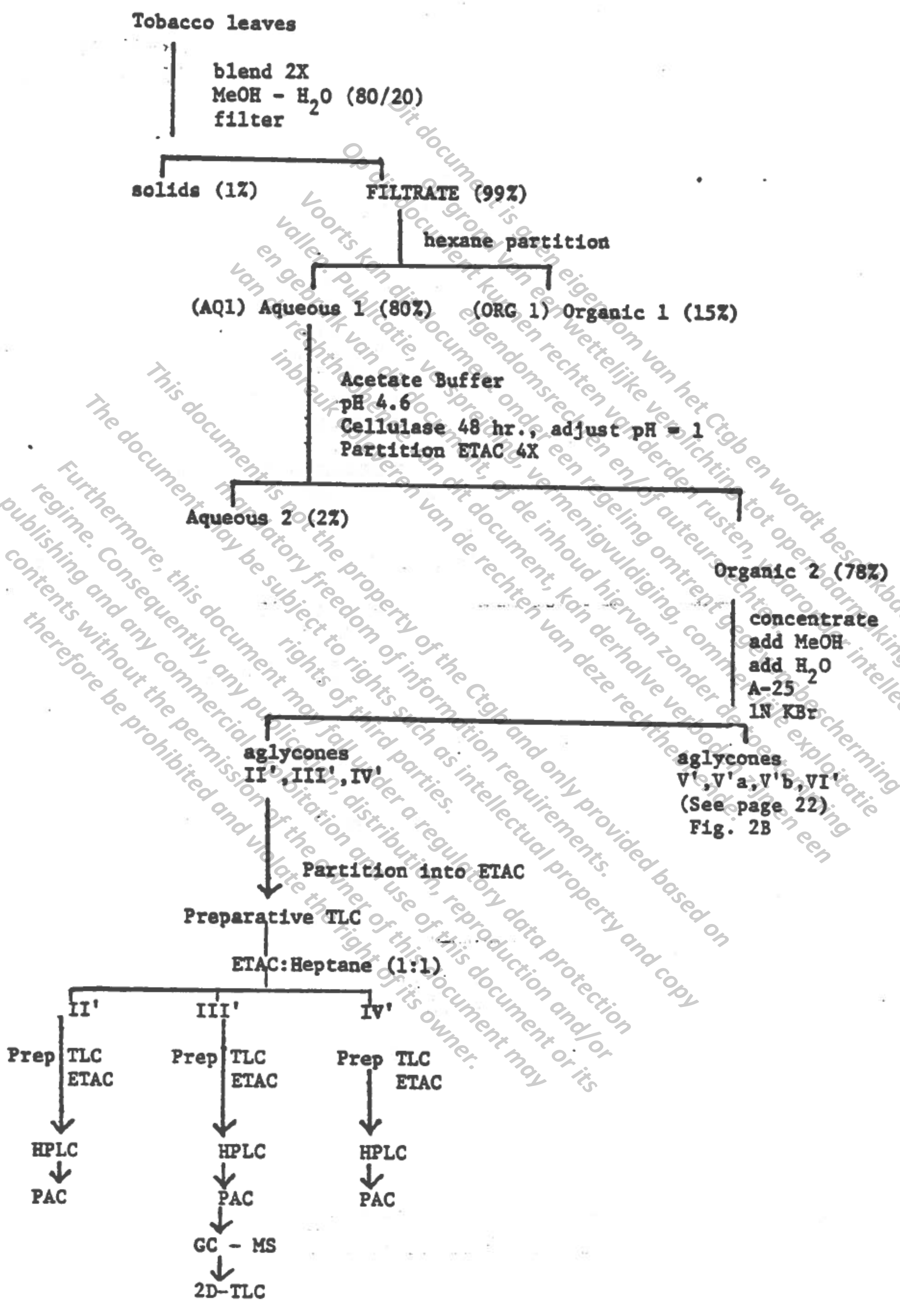

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FIGURE 2A: <u>ISOLATION AND PURIFICATION OF AGLYCONES OF ϕ-^{14}C-CGA-48988 POLAR METABOLITES IN CURED TOBACCO LEAVES</u>

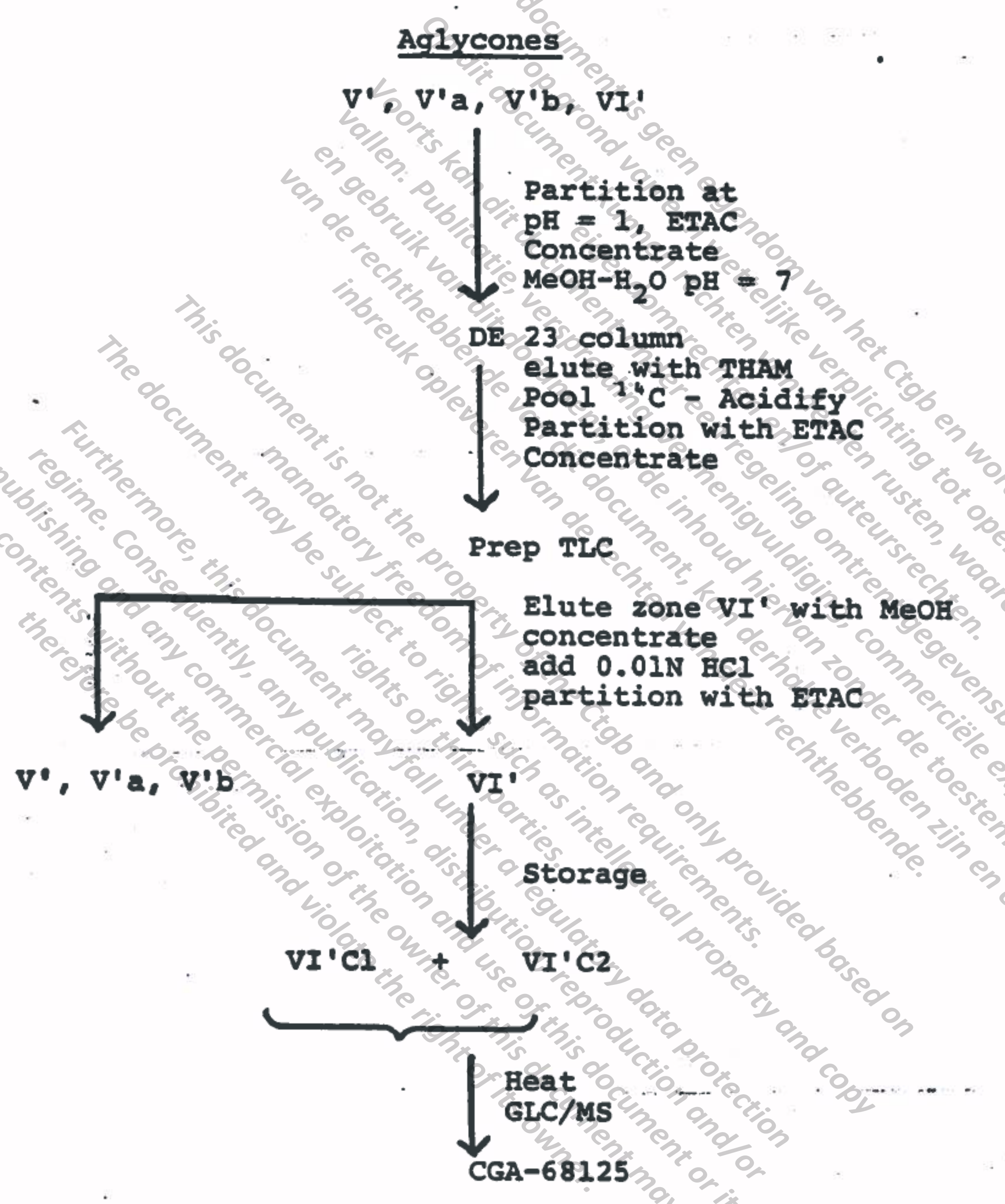

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FIGURE 2B: PURIFICATION OF ^{14}C-AGLYCONE VI' FROM CURED TOBACCO LEAVES

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FIGURE 3: X-RAY FILM OF 2D-TLC OF AGLYCONES OF ϕ-^{14}C-CGA-48988 METABOLITES IN TOBACCO LEAVES. SEE TABLE I.

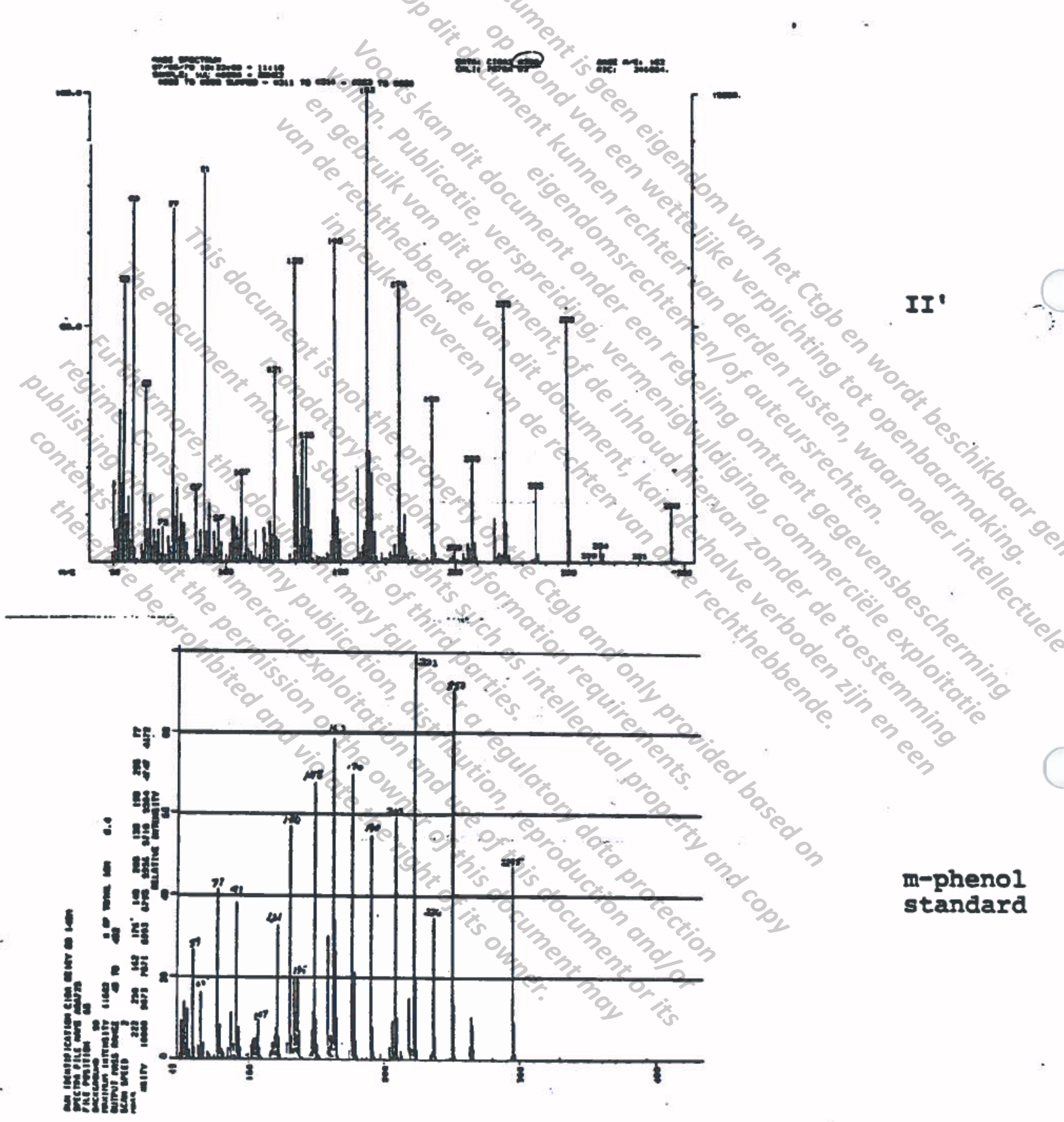

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FIGURE 4: GLC/MS - E.I. SPECTRA OF AGLYCONE II' FROM TOBACCO LEAVES AND SYNTHESIZED M-PHENOL OF CGA-48988

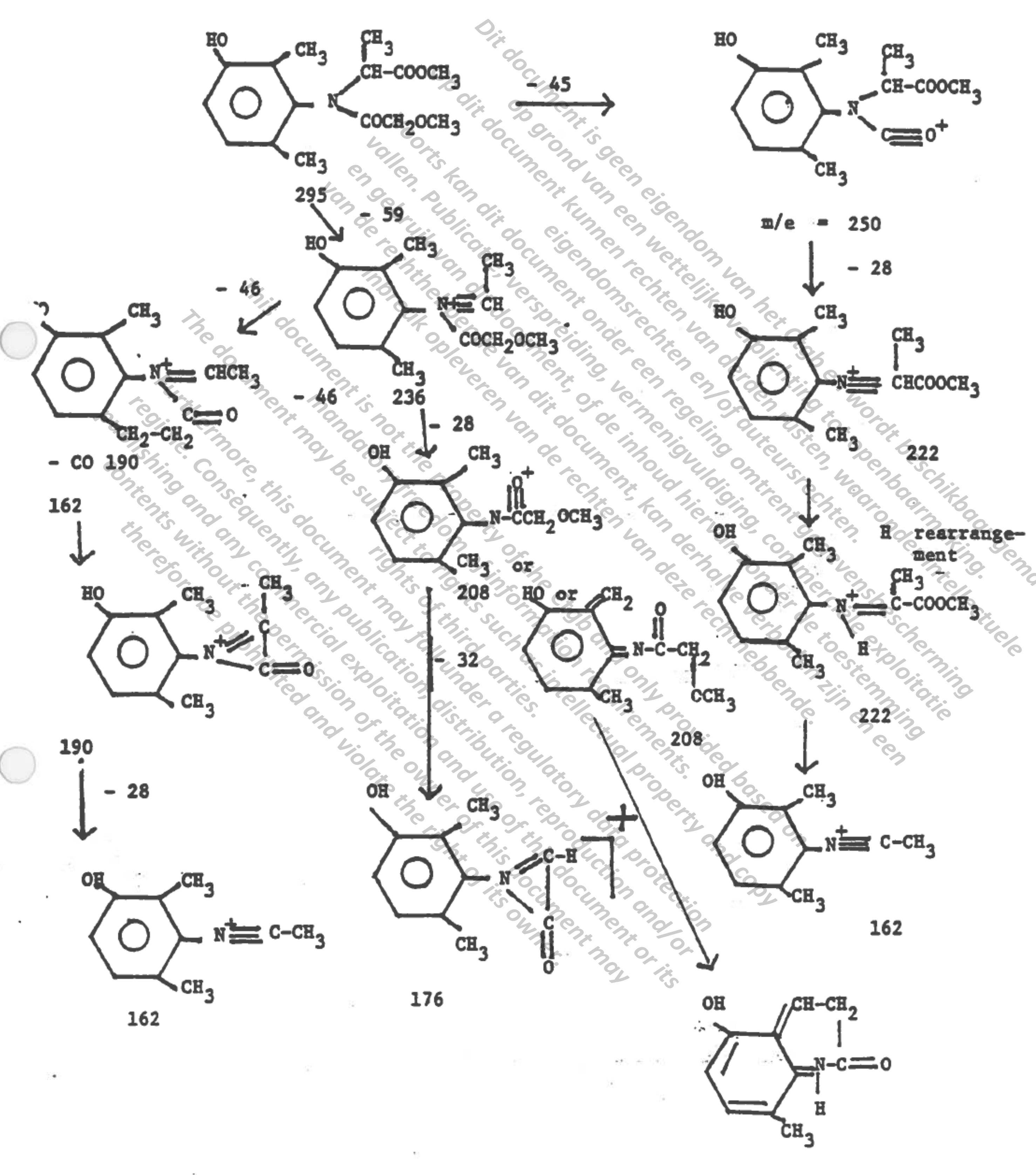

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FIGURE 5: POSSIBLE FRAGMENTATION PATTERN OF II' (REFERENCE 7)

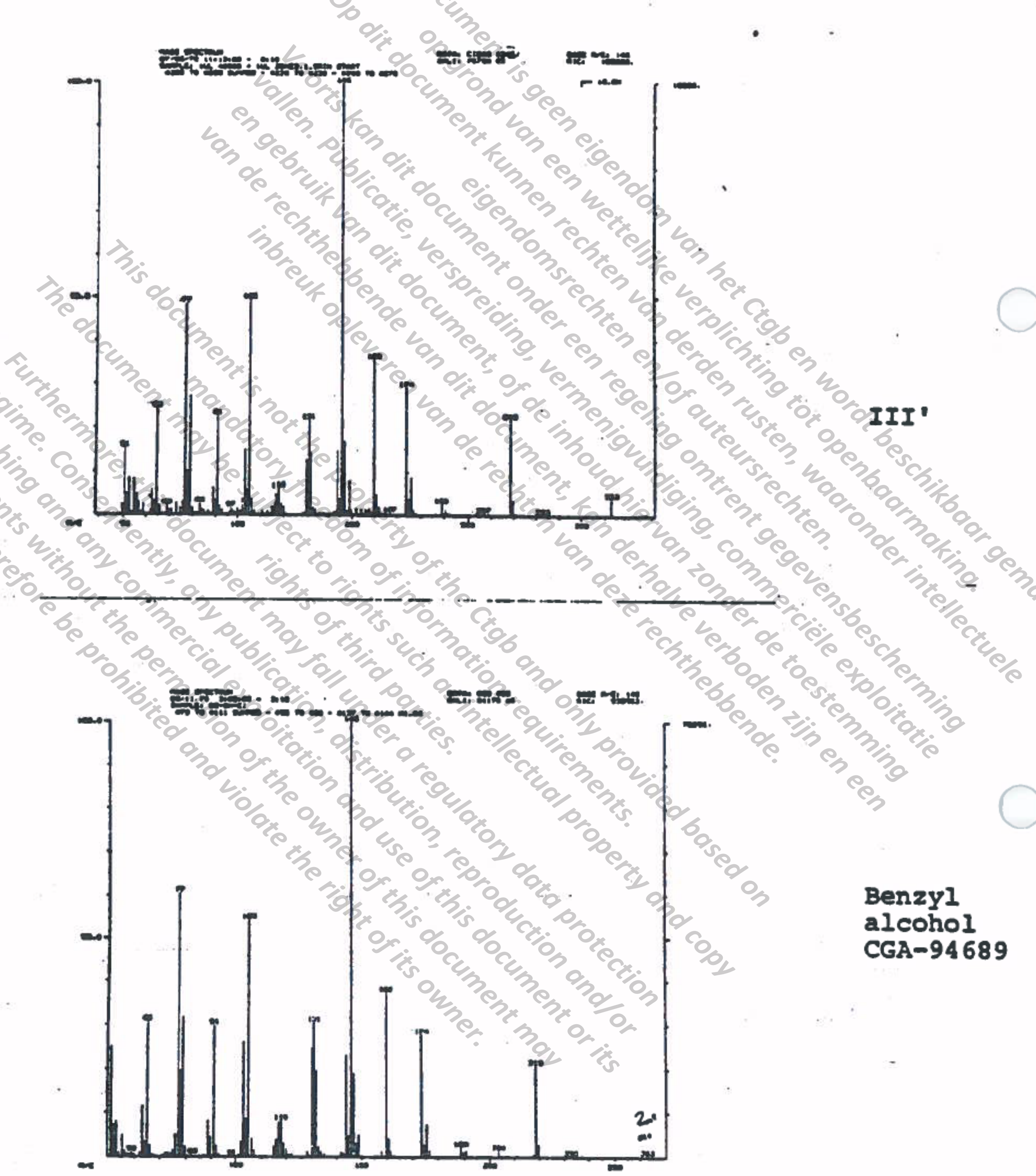

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FIGURE 6: GLC/MS - E.I. SPECTRA OF THE AGLYCONE III' AND OF THE SYNTHESIZED BENZYL ALCOHOL OF CGA-48988 (ISOMER A, CGA-94689)

FIGURE 7: POSSIBLE FRAGMENTATION PATTERN OF III' OR IV' (REFERENCE 7)

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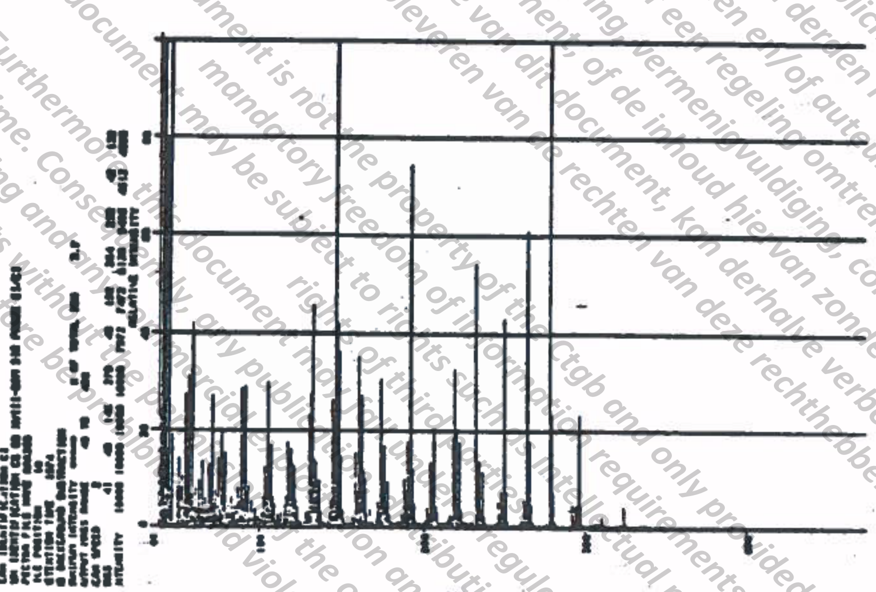

FIGURE 8: C.I. MASS SPECTRUM OF BENZYL ALCOHOL OF CGA-48988, SOLID PROBE. C.I.

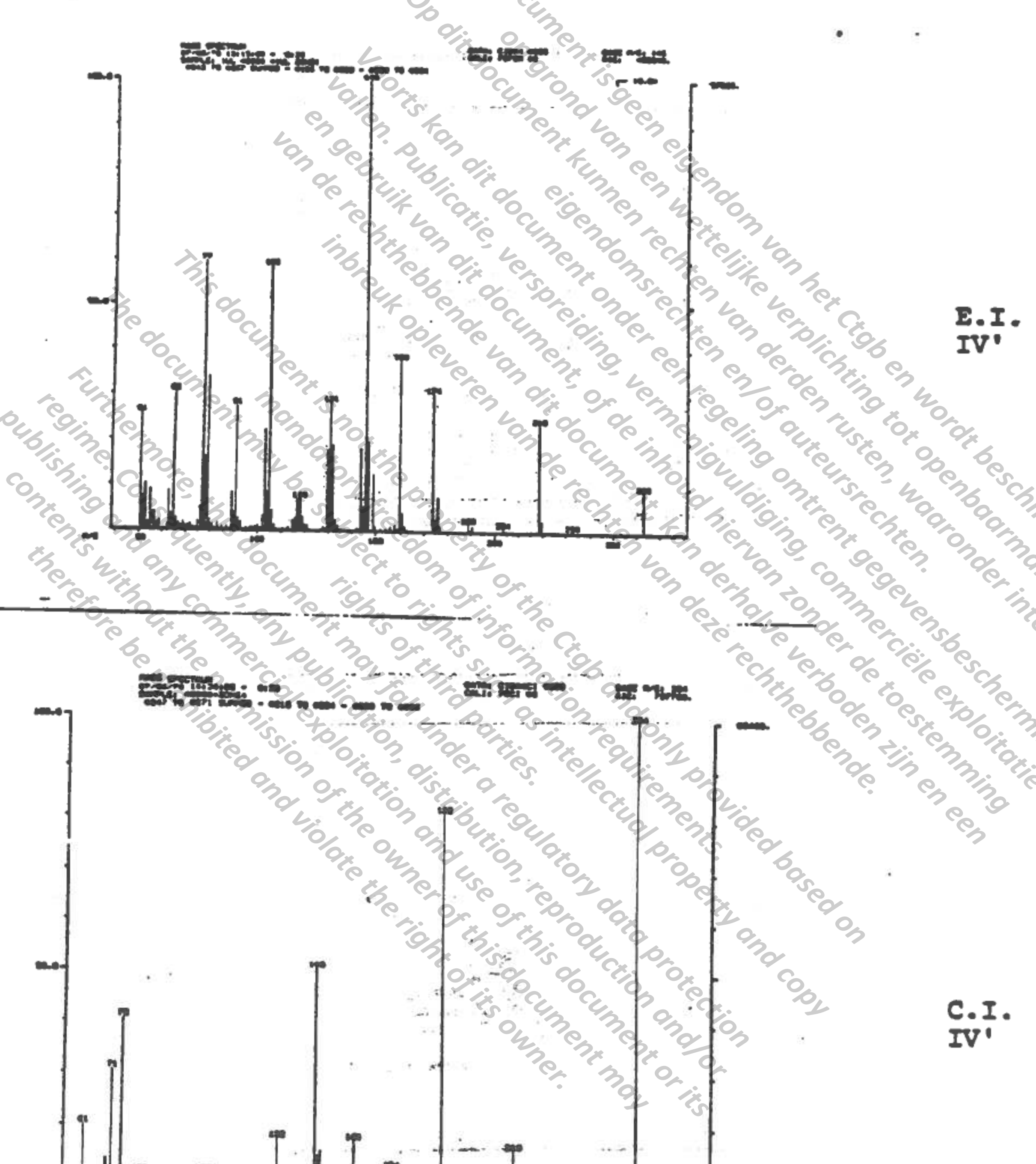

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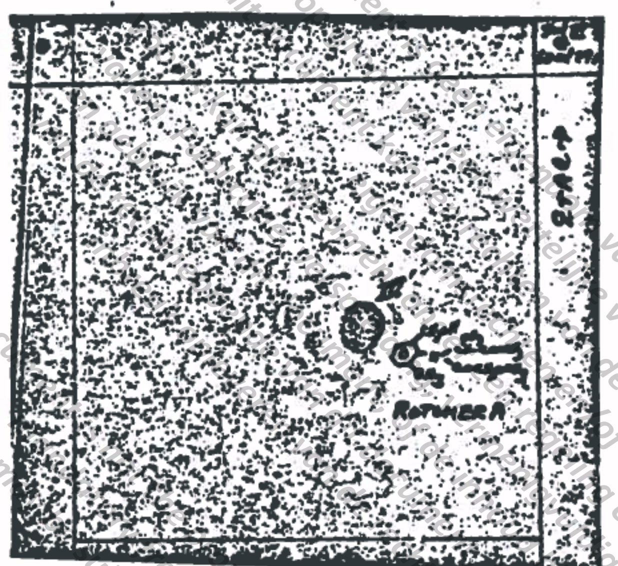
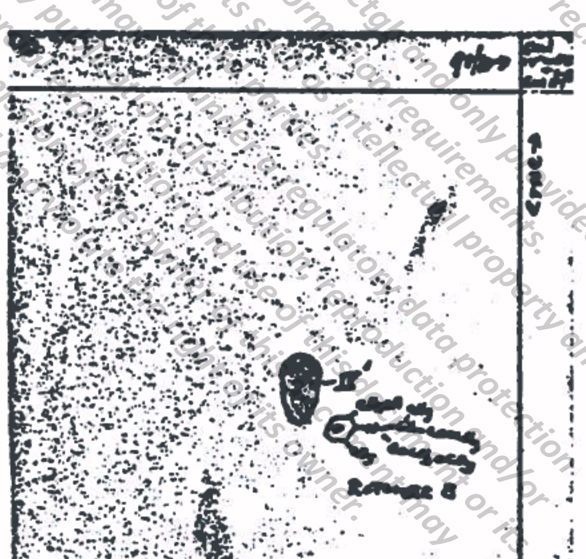
FIGURE 9: GLC/MS - E.I. AND C.I. OF AGLYCONE IV'



III'



IV'

FIGURE 10: <u>AUTORADIOGRAMS OF TWO DIMENSIONAL THIN LAYER CHROMATOGRAPHY OF AGLYCONE III' VS. BENZYL ALCOHOL ATROPISOMER A AND AGLYCONE IV' VS. BENZYL ALCOHOL ATROPISOMER B</u>

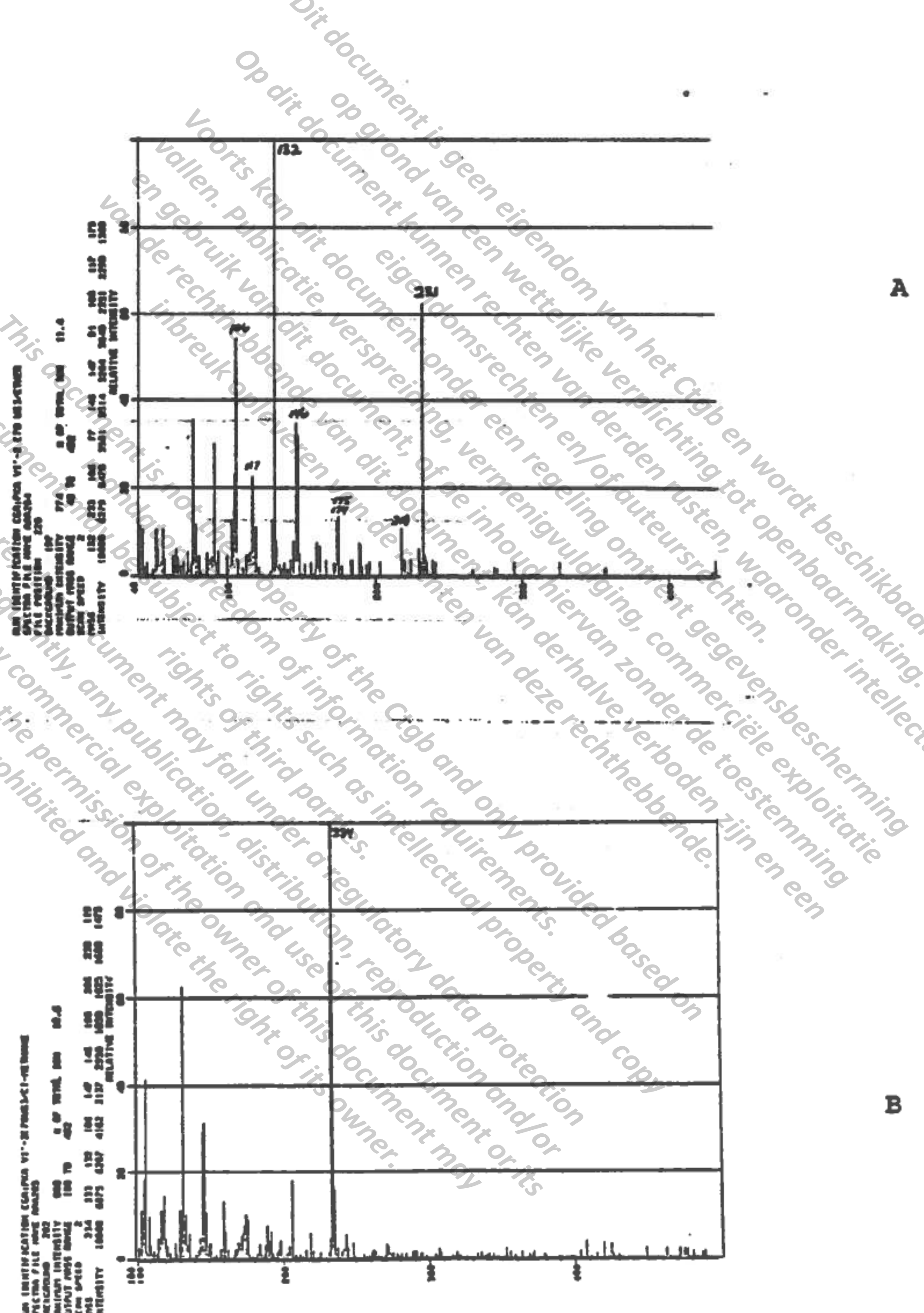


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FIGURE 11: GLC/MS OF AGLYCONE VI'. (A) E.I. GLC MASS SPECTRUM OF AGLYCONE VI'C1 + VI'C2; (B) C.I. GLC MASS SPECTRUM OF AGLYCONE VI'C1 + VI'C2; (C) E.I. GLC MASS SPECTRUM OF CGA-68125

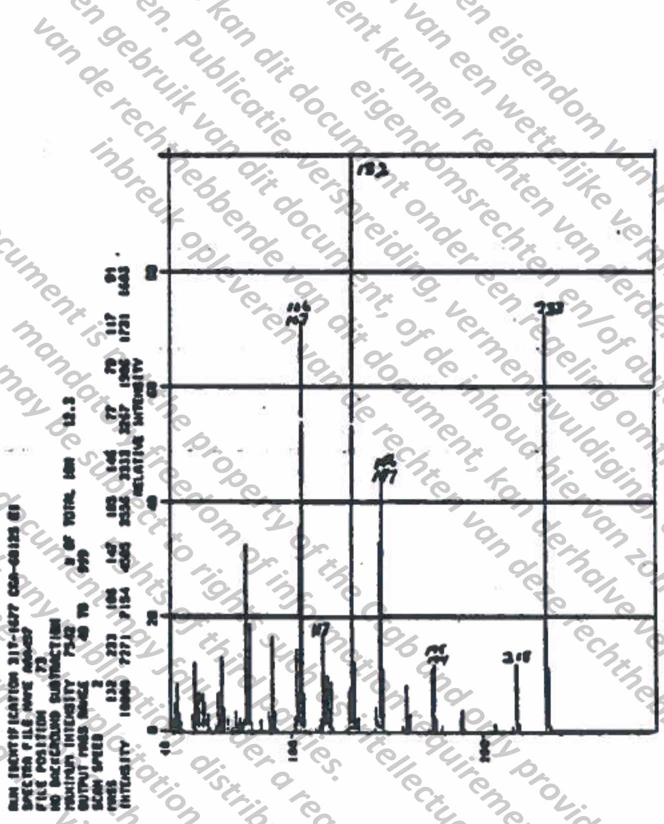

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FIGURE 11: (Continued)

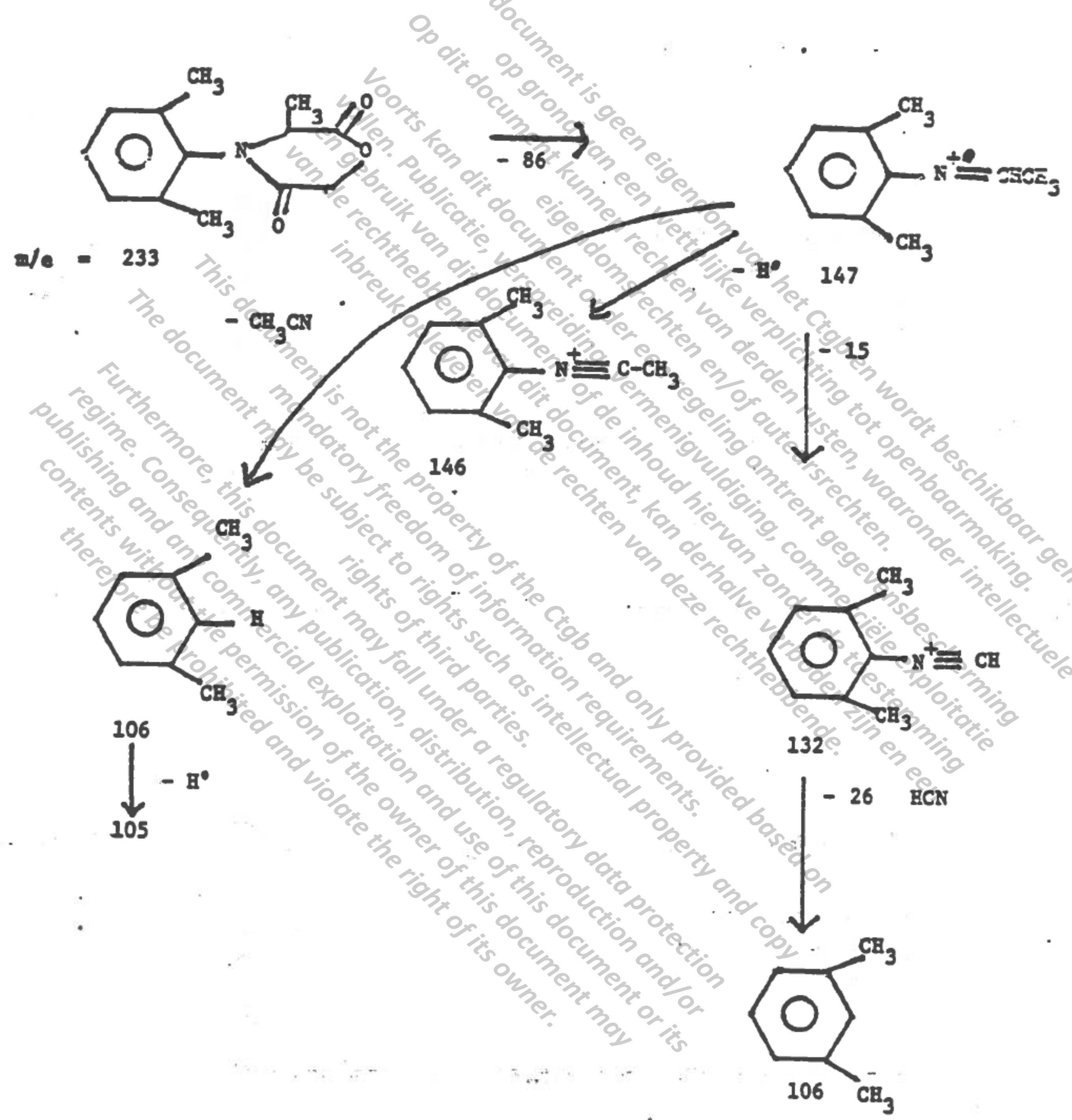

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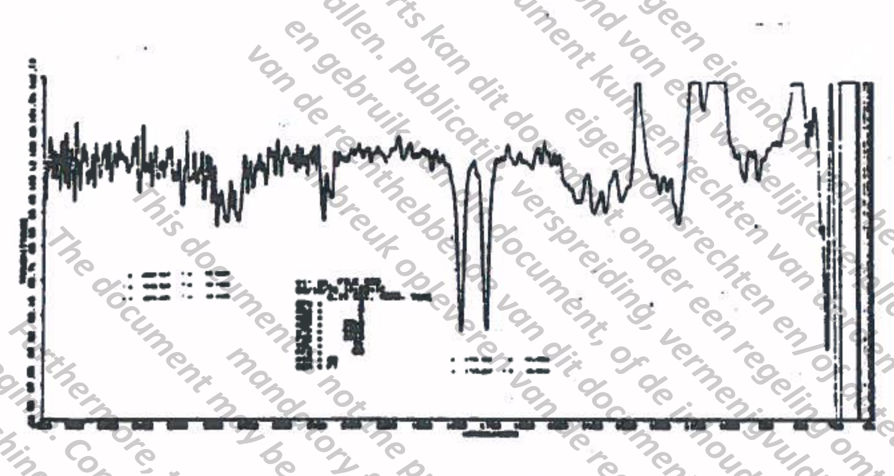
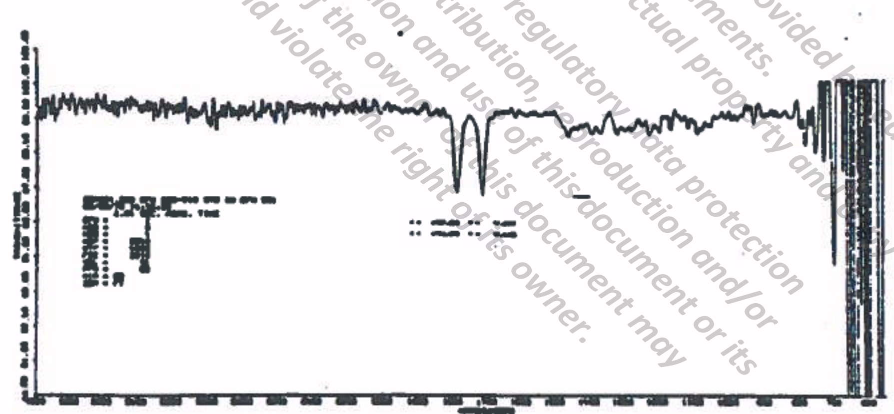
FIGURE 12: POSSIBLE FRAGMENTATION OF VI' - CGA-68125 (REFERENCE 7)



VI' C1
+
VI' C2



CGA-68125

FIGURE 13: COMPARISON OF GLC/FTIR SPECTRA FOR AGLYCONE VI'C1 + VI'C2 AND CGA-68125

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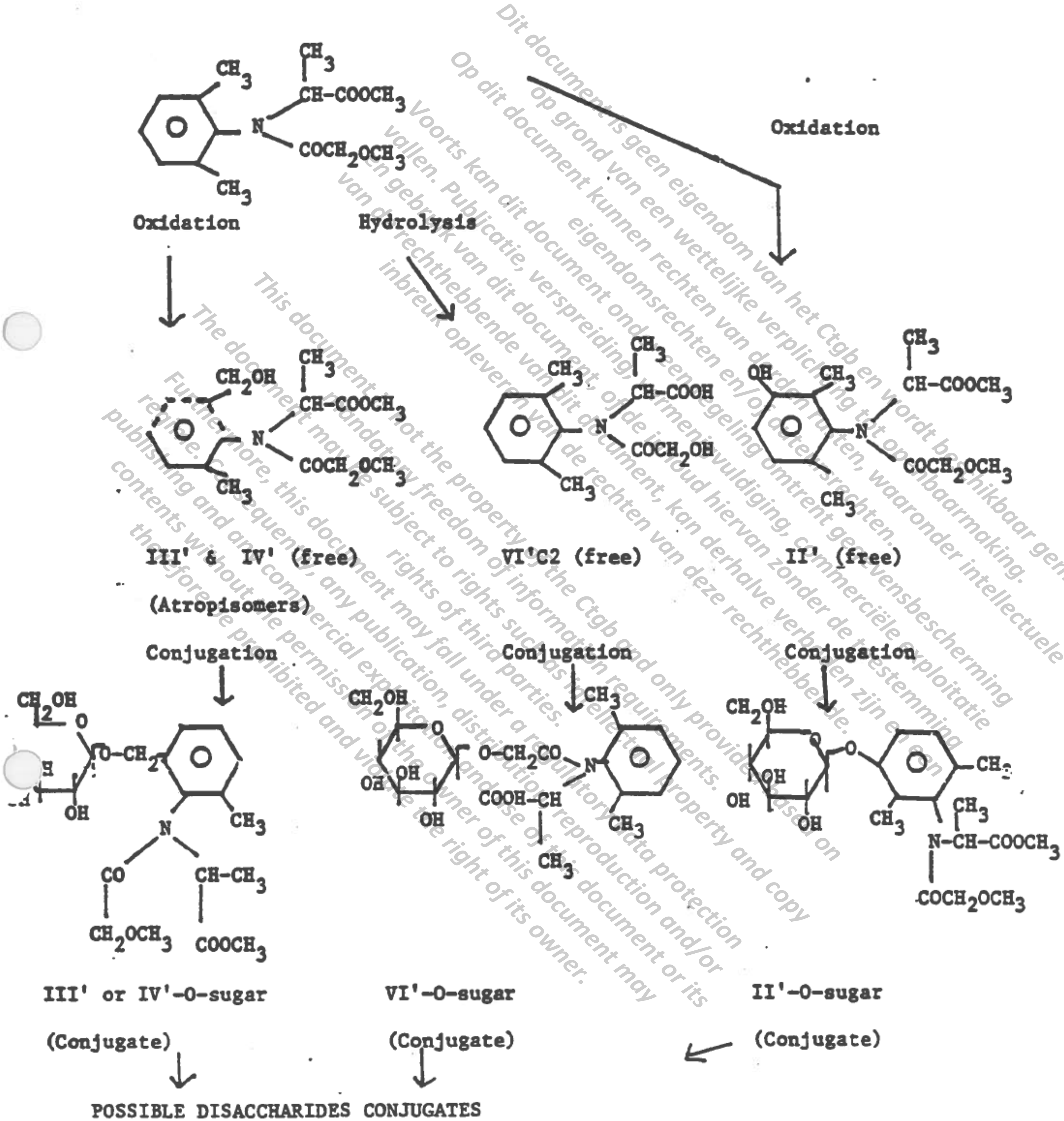

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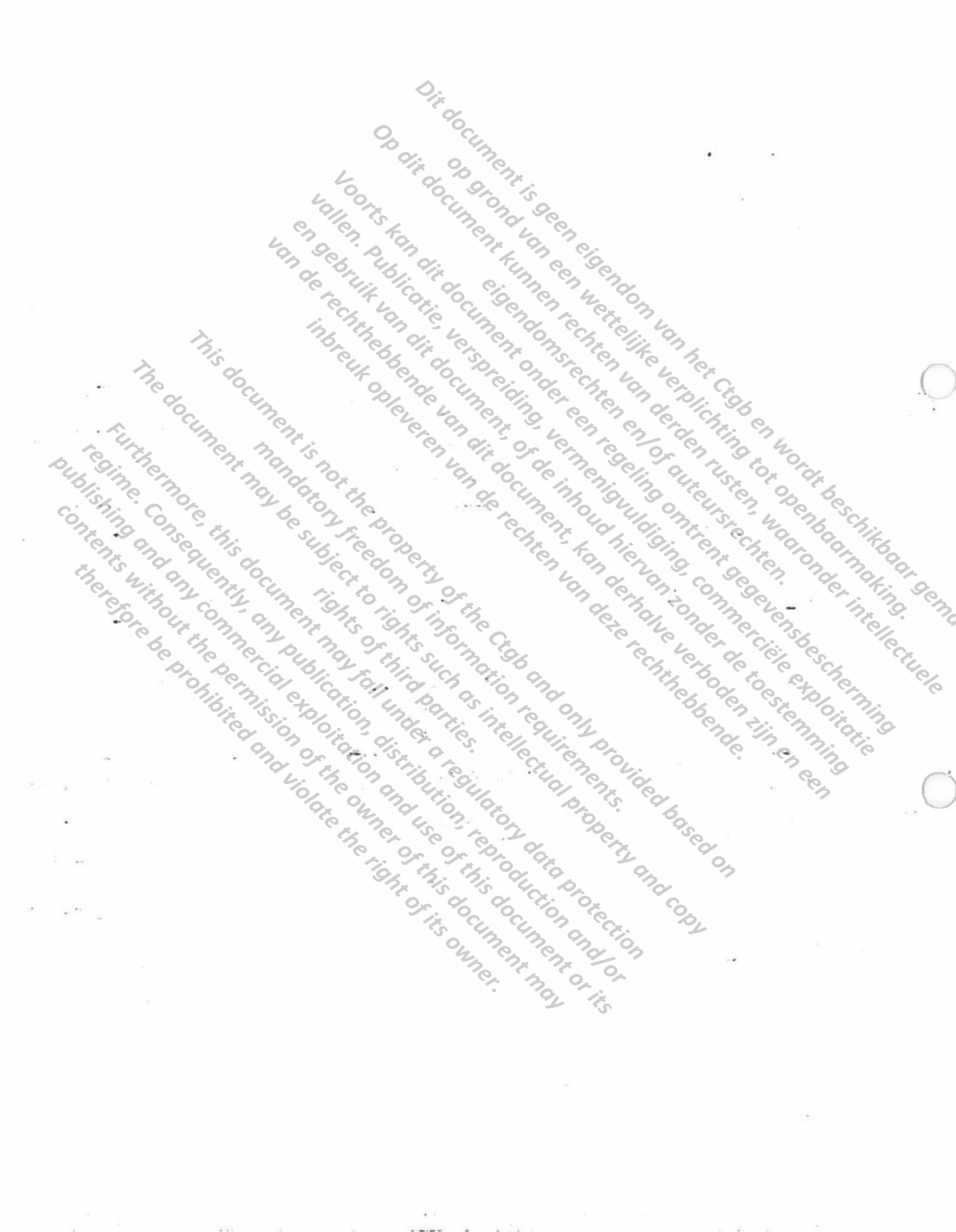
FIGURE 14: PROPOSED PATHWAYS FOR METABOLISM OF CGA-48988 IN TOBACCO LEAVES



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