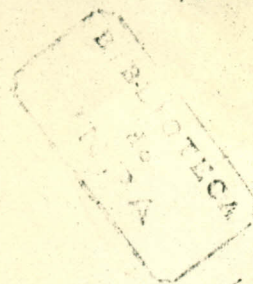


12 March 1978



INVESTIGATIONS OF THE LATEX OF AMAZONIAN TREES: *Massaranduba* *Verdadeira* -  
*Manilkara* *surinamensis* (Mig.) Dubard (P) (Sapotaceae) and *Sorva* da Mata (or  
*Sorva* Grande) - *Couma* *macrocarpa* Barb. Rodr. (Apocynaceae)

by

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BRASIL

Investigations of the latex ...  
1978 FL-FOL3835



CPAA-18923-1

FOL  
3835



## INTRODUCTION

The latex samples were harvested by cutting the trunk of the named trees located in Ducke Reserve (INPA property, Manaus-Amazonas) at the beginning of the wet season in the first week of October, 1977, and stored in screw cap vials at room temperature. The samples did not suffer obvious oxidation. However, a third latex sample, harvested at the same time and same place of the tree Leiteira was not analysed because it suffered marked deterioration when exposed to air and light, going from white to pink at harvest and then to red-brown on further handling.

## EXTRACTION

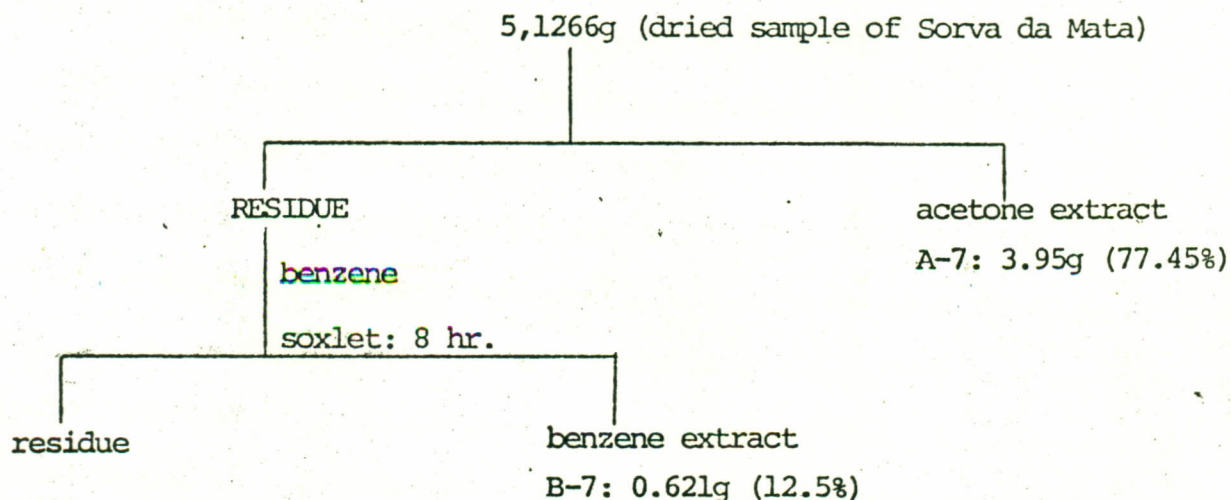
Small amounts of the samples were weighed, put in crystallization dishes, and left to dry in a dessicator with anhydrous  $\text{CaSO}_4$  until constant weight. The yield of dry material was: massaranduba - 70%, and Sorva da Mata - 50%.

The dried samples were extracted in a soxlet apparatus with heptane, acetone and benzene successively (4 hours each solvent) and the extracts evaporated to dryness and weighed. The yields as percent of dry weight are given in the table below:

Percent Yields of Latex Extractions (based on dry weight)

	heptane extract	acetone extract	benzene extract
massaranduba verd.	30 (H-9)	30 (A-9)	15 (B-9)
sorva da mata	60 (H-11)	20 (A-11)	(B-11)

Trials involving initial extract with acetone (scheme I below) resulted in high percent extractions (>75%).



Initial extraction with nonpolar solvent results in a relatively insoluble residue (only partially soluble in  $\text{CHCl}_3$ ). High percent extractions with heptane is thought to be the result of naturally occurring surfactants.

Comparison by TLC on silica gel (Baker) of the heptane and acetone extract of both latices show 3 spots. These and the  $R_f$  values of the standards used are shown in Table I. (The plates were sprayed with rhodamin and the spots revealed by  $\text{I}_2$  vapours and UV light).

Table I. TLC data on Latex Extracts and Standards

	$R_f$ Values		
Heptane extracts (H-9, H-11)	0.58	0.40	0.12
Acetone extracts (A-9, A-11)	0.58	0.40	0.12
free sterols (cholesterol, lanosterol + $\beta$ -sitosterol)	--	--	0.11
cholesterol acetate	--	0.40	--
cholesterol oleate	0.58	--	--
solvent system (petroleum ether-diethyl ether-glacial acetic acid, 90:10:1)	--	--	--
			0.93



Nmr spectra of samples H-9, H-11, A-9 and B-7 were obtained; i.r. spectra of H-9 and H-11 were also recorded. Both i.r. spectra show an ester group absorption at  $1730\text{ cm}^{-1}$  (probably an acetyl), and the absence of an OH absorption in the  $1000\text{ cm}^{-1}$  to  $3050\text{ cm}^{-1}$  region.

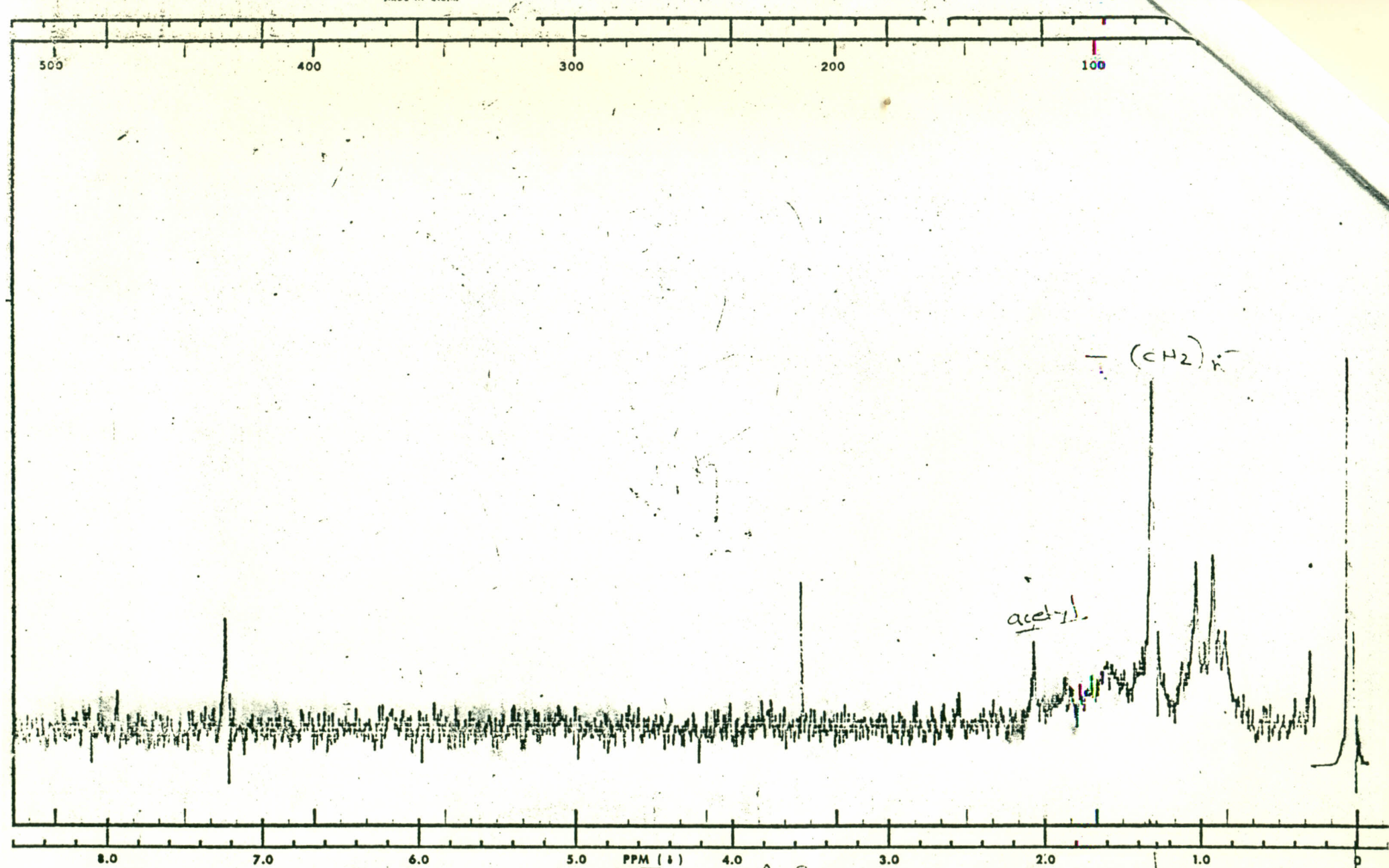
The nmr spectra of the heptane and the acetone extracts were all similar, consisting of a complex multiplet centered 1.0 $\delta$ , a singlet at 1.27 $\delta$  ( $-\text{CH}_2-$ ) and a singlet at 2.0 $\delta$  ( $\text{CH}_3-\text{C}-$ ). <sup>The heptane extract spectra also show a multiplet at 5.15 $\delta$</sup>  The nmr spectrum of sample B-7 is consistent with the reported spectrum of polyisoprenes (singlet at 1.6 $\delta$  ( $\text{CH}_3$ ) doublet at 2.0 $\delta$  ( $-\text{CH}_2-$ ), multiplet at 5.0 $\delta$  (vinyl)). Some unidentified impurities are also present in this sample.

The underivitized extracts were examined by gas chromatography on a Perkin Elmer Model 900 equipped with flame ionization detector (FID). The column employed was a Silanox type glass open tubular column coated with SE-30 at  $260^\circ\text{C}$  with helium as carrier gas (5ml/min). A dry injection system constructed according to Van der Berg and Cox was employed.<sup>1</sup> The chromatograms obtained for each extract were markedly similar with two major constituents in the same ~~xxx~~ relative proportions and 2-3 minor components. Figure 1 shows a typical chromatogram (heptane extract of massaranduba: H-9) and retention index data for each extract are summarized in Table 2.

Table 2. Retention Index Data for Latex Extracts

	H-9	A-9	H-11	A-11
a	3340	—	3350	—
b	3350	3350	3355	3355
c	3360	—	3360	3370
d	3385	3390	—	3385
e	3395	—	3400	3405
f	—	—	—	3420
g	3460	—	3480	—





MANUAL ☒ AUTO ☐

SWEEP OFFSET (Hz): ----- SWEEP TIME (SEC): 

50	250
----	-----

 (250)

SPECTRUM AMPLITUDE: ----- SWEEP WIDTH (Hz): 

25	50	100	250	500
----	----	-----	-----	-----

 (500)

INTEGRAL AMPLITUDE: ----- FILTER: 

1	2	3	4	5	6	7	8
---	---	---	---	---	---	---	---

 ( 2)

SPINNING RATE (RPS): ----- RF POWER LEVEL: ----- (.05)

SAMPLE: A-9 dried. REMARKS: 1.27

SOLVENT: CDCl<sub>3</sub>



500

400

300

200

100

8.0

7.0

6.0

5.0

PPM (τ)

4.0

3.0

2.0

1.0

0

MANUAL

AUTO ☐

SAMPLE: A-9

REMARKS:

SWEEP OFFSET (Hz):

SWEEP TIME (SEC):

50 150

(250)

SPECTRUM AMPLITUDE: 10x25

SWEEP WIDTH (Hz):

25 50 100 250 500

(500)

INTEGRAL AMPLITUDE:

FILTER:

1 2 3 4 5 6 7 8

(2)

SPINNING RATE (RPS): 37

RF POWER LEVEL: 0.03

(.05)

SOLVENT: CCl<sub>3</sub>acetone extr. of  
massachusetts. (2 solvent cycles)varian  
analytical instrument division

DATE:

12/15/77

OPERATOR:

EKN

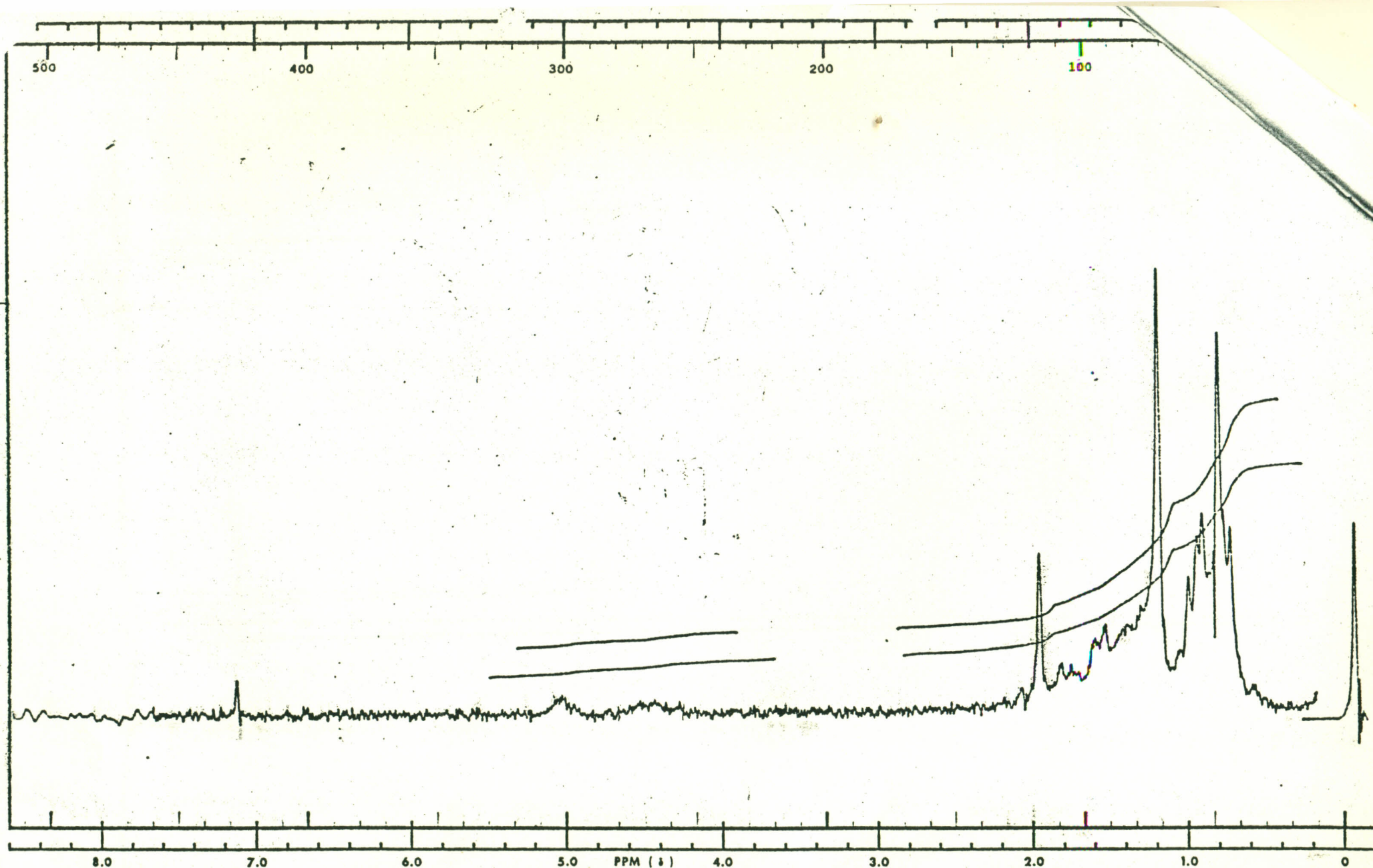
60 MHz NMR

G

SPECTRUM NO.

52156





SWEEP OFFSET (Hz): 10x2  
 SPECTRUM AMPLITUDE: 10x2  
 INTEGRAL AMPLITUDE: -----  
 SPINNING RATE (RPS): -----

MANUAL ☒ SWEEP TIME (SEC): 50  
 SWEEP WIDTH (Hz): 25 50 100 250  
 FILTER: 1 2 3 4 5 6 7 8  
 RF POWER LEVEL: -----

AUTO ☐ (250)  
 (500)  
 ( 2)  
 (.05)

SAMPLE: 4-9

SOLVENT: CDCl<sub>3</sub>

REMARKS: -----



varian  
 analytical instrument division

DATE: 1/18/78

OPERATOR: NEVES

60 MHz NMR  
 SPECTRUM NO. -----

G

53301

Fig. 1

H-9~

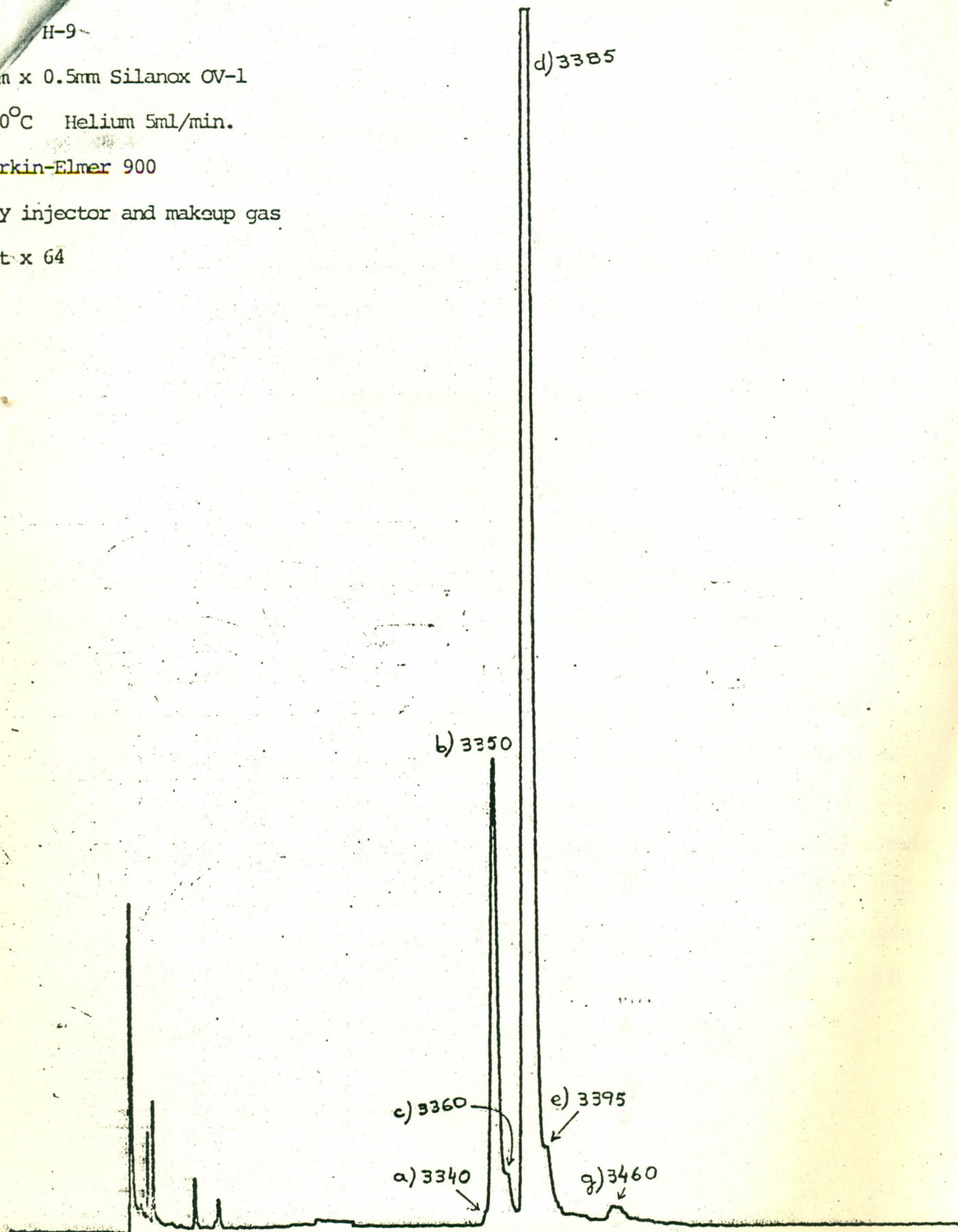
n x 0.5mm Silanox OV-1

260°C Helium 5ml/min.

Perkin-Elmer 900

Dry injector and makeup gas

Att x 64





The spots of heptane and acetone extracts (H-9, H-11, A-9, A-11) were further separated by preparative TLC, heptane and ethyl acetate (20:1) as eluent. Three fractions were isolated in each case: F-1 (low polarity), F-2 (intermediate polarity), F-3 (high polarity) and F-4 (origin).

These fractions were reexamined by gas chromatography (15m x 0.2mm WCOT SE-52, 230°C) and in each case the behavior of samples from each extract were identical. Fractions F-1 showed no materials chromatographable under the conditions employed. Fractions F-3 showed two very broad peaks and were not further investigated. Materials from the origin (F-4) were not examined.

Fractions F-2 were identical in GC behavior to the crude fractions. Under the new GC conditions a higher efficiency was obtained (35,000 theoretical plates) and an additional major constituent was resolved. A typical chromatogram is shown in Fig. 2.

Combined gas chromatography-mass spectrometry (DuPont 29-291/2, 70m x 0.2mm WCOT SE-52, 260°C, source temperature 240°C, electron 70eV) revealed that the three peaks were isomeric with mass spectral features consistent with that of mono-hydroxy pentacyclic triterpenes as their acetates. The mixture was likewise examined by high resolution mass spectrometry (CEC-110, 150°C, resolution 1:10,000). The molecular ion  $m/e$  468 was shown to have the formula  $C_{32}H_{52}O_2$  and the base peak,  $m/e$  218,  $C_{16}H_{26}$  further supporting this hypothesis. Figure 2 shows the low resolution mass spectrum of the unresolved mixture H9,F2.



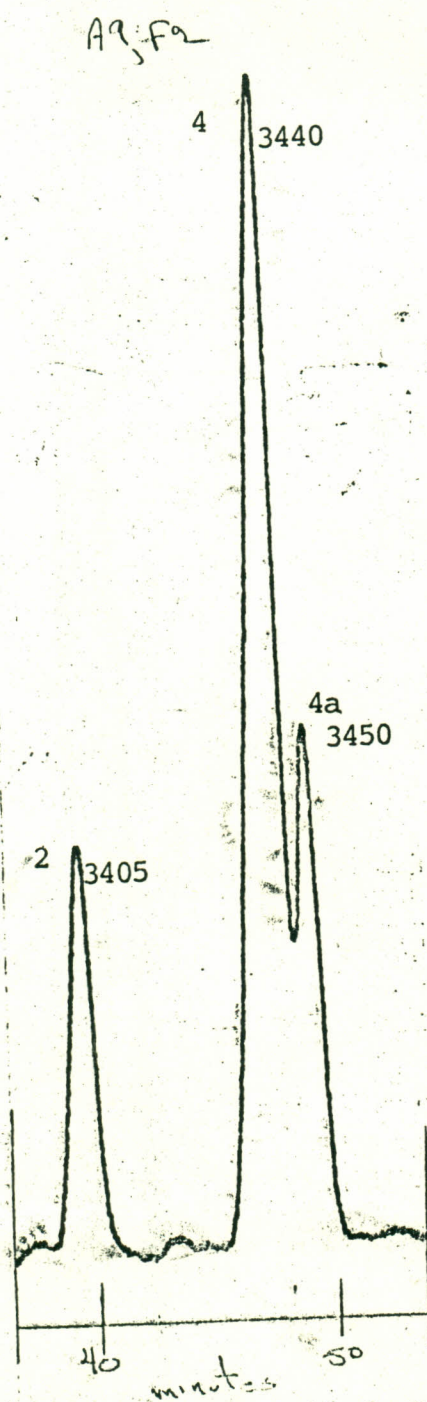
Figure 2

15m x 0.2mm WCOT SE52

230°C

Helium carrier gas 2ml/min

Varian Aerograph 2700





EXPAND BY 80 ABOVE MASS 80  
 BASE 14375 \*2\*\* 8 % TOTAL IONIZ 6

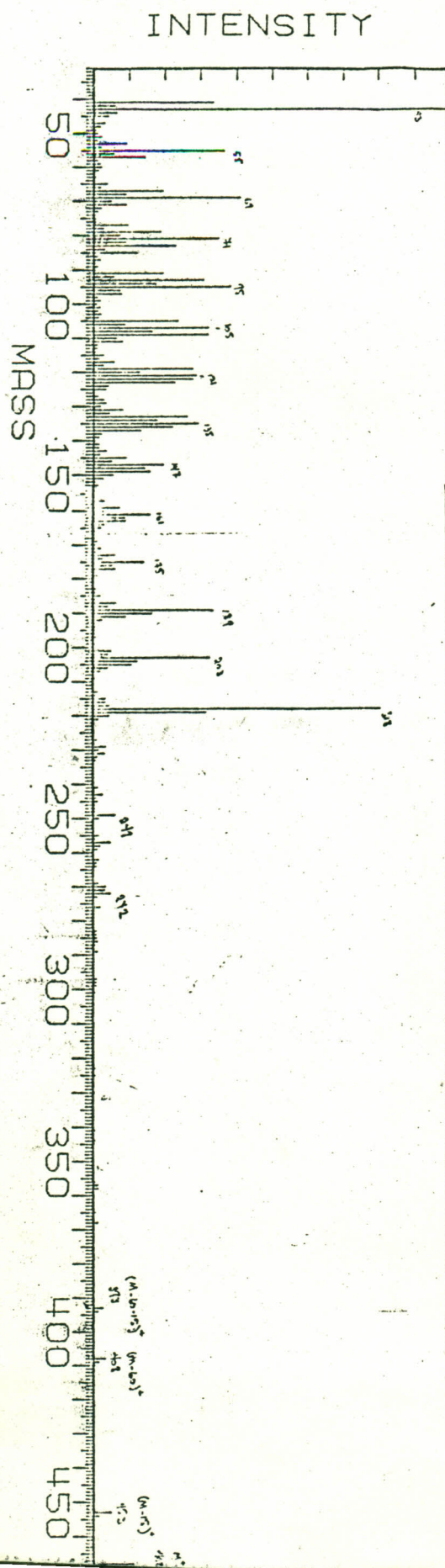


Figure 1

H<sub>2</sub>SO<sub>4</sub>  
 D<sub>2</sub>O  
 20%