order of magnitude of k_r as 10^3 s^{-1} at 290 K. Experiments to measure k_r directly using time-resolved ESR. spectroscopy are in progress.

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77. Studies on the Constituents of Capsicum. Part 3[1]

A Quantitative Determination of Olefinic and Saturated Components by NMR. and of *trans*-Components by IR. Spectroscopy in a Capsaicinoid Mixture from Natural Source

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(12. I. 73)

Introduction. – The crystalline pungent principle of *Capsicum annuum* is the mixture of capsaicin, dihydrocapsaicin and their homologs [1] [2]. We have already described the separation of these components by gas-chromatography [2] and their quantitative estimation in the mixture [3]. In the present work we report appropriate

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[1] For part 1 & 2 see [2] & [3].

methods for a direct determination of olefinic and saturated components by NMR. as well as of *trans*-configurated components by IR. in such isolated capsaicinoid mixtures, thus avoiding the tedious separation described in [1].

Experimental. – The NMR.-spectra were recorded at 36° on a *Varian* A-60A Spectrometer using samples with 20 mg for 60 MHz and 10 mg for 100 MHz of the mixture in deuterated chloroform. Trimethylsilane (TMS) was used as internal standard. The IR.-spectra of synthetic capsaicin (CSyM-70) and capsaicinoid mixture (CGF-69) in chloroform were recorded on *Beckman* Infrared spectrometer IR 5. The area of absorption band at 970 cm^{-1} was measured by *Hewlett Packard* Digitizer.

Capsaicinoid mixtures[2]: CGM-69, CGF-65, CGF-69, CGF-70, CGF-71 and synthetic capsaicin (CSyM-70) were used.

Calculation of olefinic and saturated fractions: In 60 MHz spectra of the mixture, the peaks were designated a, b for the doublet corresponding to olefinic and a′, b′ to saturated components (where b and a′ overlaps). The ratio b/a for synthetic capsaicin (CSyM-70) was found to be 0.857.

$$\% \text{ olefinic fraction in the mixture} = \frac{I_a + (I_a \cdot 0.857)}{I_t} \cdot 100$$

I_a = integration of peak a

I_t = total integration of all the three peaks in the region of 0.8 – 1.0 ppm

In case of 100 MHz spectra:

$$\% \text{ olefinic fraction} = \frac{I_u}{I_u + I_s} \cdot 100$$

Where I_u and I_s are integrations of doublets corresponding to olefinic and saturated fractions respectively

$$\% \text{ saturated fraction} = 100 - \% \text{ olefinic fraction.}$$

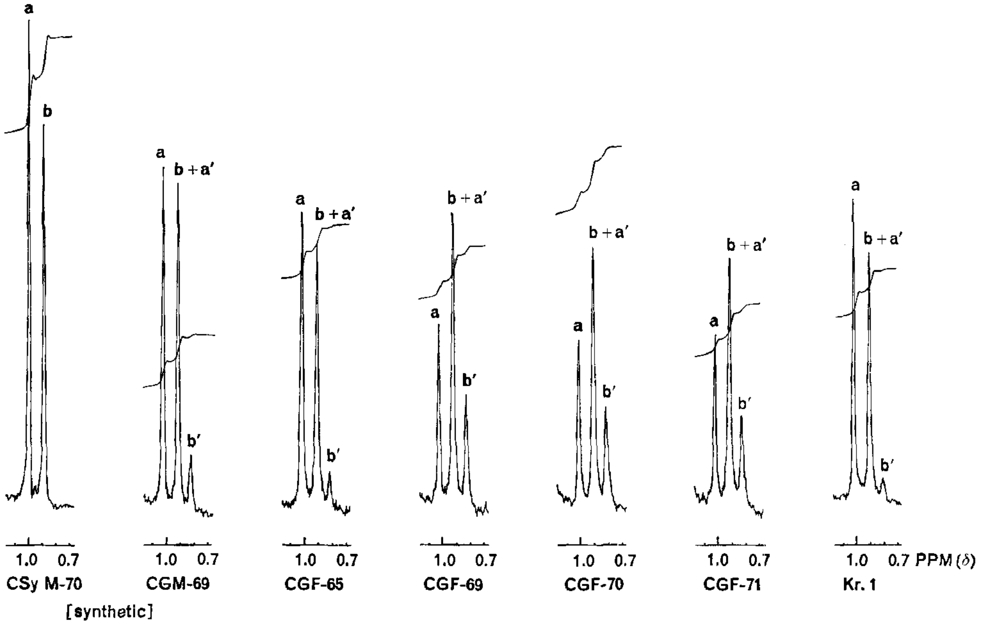

Fig. 1. *The* $[C(CH_3)_2]$ *absorbances in 60 MHzNMR.-spectra of different capsaicinoid mixture in* $CDCl_3$

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[2] For sources see [2].

Results and Discussion. – *Estimation of olefinic and saturated components in the capsaicinoid mixtures*: The NMR.-spectra of capsaicin and dihydrocapsaicin differ distinctly in the high field resonance between 0.8–1.0 ppm. It is observed that the 6 dimethyl protons of isopropyl group in these spectra appear as doublets centered at 0.96 ppm for capsaicin and 0.87 ppm for dihydrocapsaicin. The 60 MHz spectra of natural capsaicinoid mixtures are inconsistent with that of the synthetic capsaicin in the above mentioned field (Fig. 1). Three lines arising in this region (0.8–1.0 ppm) suggest that they resulted from the two doublets for dimethyl protons corresponding to olefinic and saturated fractions in these mixtures by one peak overlapping. Since the difference of one carbon atom in homologs of capsaicin and dihydrocapsaicin does not practically change the chemical shift of $C(CH_3)_2$ protons, the respective doublets in the mixture are also centered at 0.96 and 0.87 ppm alike in the spectra of synthetic capsaicin and dihydrocapsaicin. The total area of absorption in this region integrates for 6 protons in case of each capsaicinoid mixture, which enabled us to calculate the per cent proportion of the olefinic and saturated components measured by $C(CH_3)_2$ resonances (Table 1).

Table 1. *Per cent of olefinic and satured fractions in different natural capsaicinoid mixtures calculated from the NMR.-data*

60 MHz							100 MHz				
					% Parts					% Parts	
Substance	I_a	$I_a \cdot 0.857$	$I_a + (I_a \cdot 0.857)$	I_t	olef.	satur.	I_u	I_s	$I_u + I_s$	olef.	satur.
CSyM-70	21.0	18.00	39.00	39.00	100.00						
CGM-69	10.0	8.57	18.57	22.4	82.90	17.10	37.0	12.5	49.5	74.75	25.25
CGF-71							22.0	21.0	43.0	51.16	48.84
CGF-70	8.2	7.03	15.23	27.2	55.98	44.02	30.5	29.5	60.0	50.83	49.17
CGF-69	6.7	5.74	12.44	21.8	57.06	42.94	17.5	18.5	36.0	48.61	51.38
CGF-65	10.7	9.17	19.87	22.3	89.09	10.91	36.0	10.0	46.0	78.26	21.74
Kr. I[a]	10.5	8.99	19.45	20.25	96.3	3.7					

[a] Capsaicinoid mixture isolated from drug.

However in the 100 MHz NMR.-spectra of these mixtures, the two sets of resonances are resolved in two distinctly clear doublets (Fig. 2).

Therefore the composition of the capsaicinoid mixtures can be determined more conveniently from the 100 MHz spectra (Table 1). It is observed from Table 1 that the composition of these mixtures varies significantly which is probably due to the difference in their isolation methods.

Trans-*Configurated components.* In the IR.-spectra of synthetic capsaicin, an isolated absorption band at 970 cm^{-1} arising from CH (out-of-plane) stretching vibrations, is specific of *trans* double-bond present in the fatty acid moiety of the molecule [4]. The spectra of chloroform solutions containing different quantity of CSyM-70 in the region of 900–1100 cm^{-1} were taken (Fig. 3) and the area of the band at 970 cm^{-1} was measured in each case (Table 2).

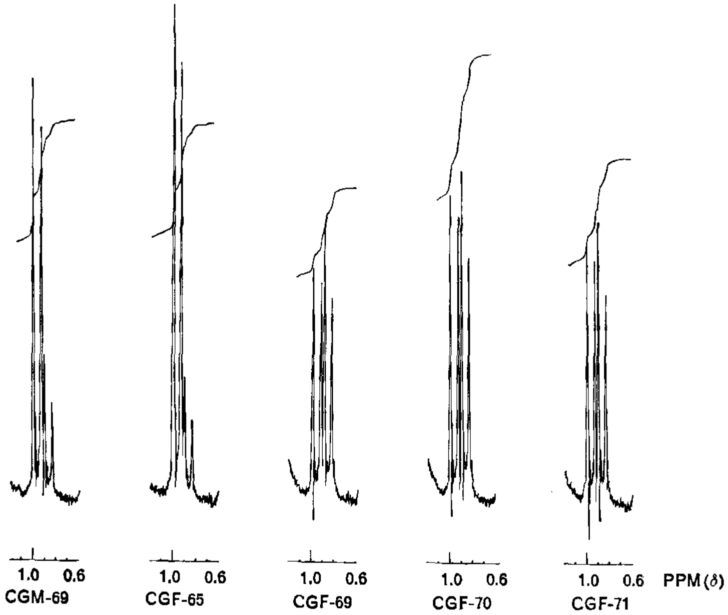

Fig. 2. *The $C(CH_3)_2$ absorbances in 100 MHz NMR.-spectra of different capsaicinoid mixtures in $CDCl_3$*

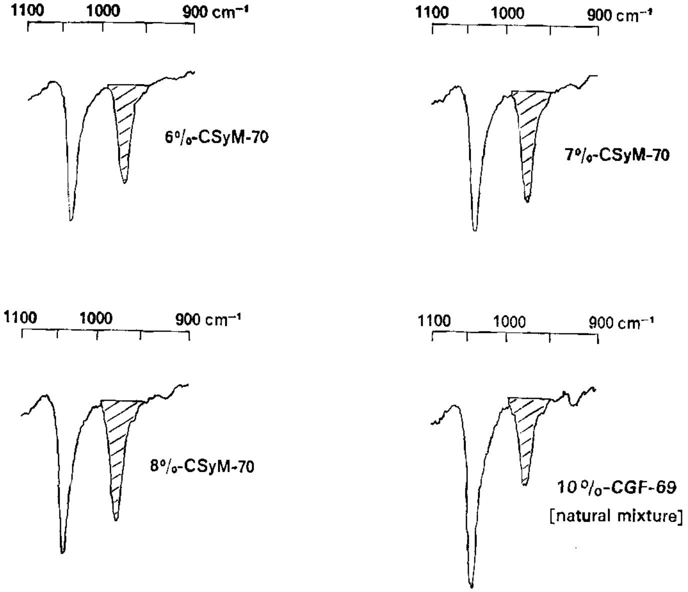

Fig. 3. *Infrared spectra of synthetic* trans-*capsaicin* (CSyM-70) *and natural capsaicinoid mixture* (CGF-69) *in the range of 1100–900 cm*$^{-1}$

Table 2. *Quantitative area data from Infrared spectra measured by digitizer*

Substance	% wt/vol	area [inch^2] band at 970 cm$^{-1\,a)}$
CSyM-70 (synth.)	6.0	0.35755
	7.0	0.41230
	8.0	0.46560
CGF-69 natural	10.0	0.33374

The value of area was plotted versus concentration (Fig. 4).

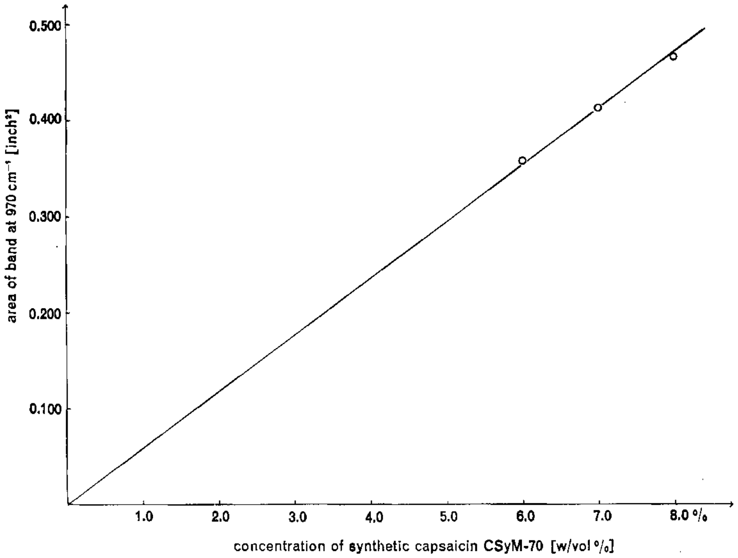

Fig. 4. *Quantitative relationship between 970 cm^{-1} band area and degree of synthetic capsaicin* (CSyM-70)

A sample with isolated capsaicinoid mixture (CGF-69) in chloroform was run in the same way. From the area obtained it was possible to determine the *trans*-components in the mixture. The analysed mixture CGF-69 was found to consist of 54% *trans*-components.

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Erratum

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