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ANALYTICAL STUDIES OF PYRETHRIN FORMULATIONS BY GAS CHROMATOGRAPHY

III. ANALYTICAL RESULTS ON INSECTICIDALLY ACTIVE COMPONENTS OF PYRETHRINS FROM VARIOUS WORLD SOURCES*

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SUMMARY

Gas chromatographic techniques were applied to studies of the insecticidally active esters of pyrethrum. Pyrethrin extracts from various world sources were compared. The greater potential importance of more definitive and precise results acquired by gas chromatography compared with results obtained by older classical procedures is illustrated and it is more evident when comparisons are made of the "true" pyrethrin I and pyrethrin II ester fractions of the extracts. Results of this more precise nature should be of increased benefit to the pesticide formulator and to the insect toxicologist.

INTRODUCTION

Pyrethrum (*Chrysanthemum cinerariaefolium* Vis), native to temperate and boreal regions[1], is grown commercially primarily in Kenya, Tanzania, Ecuador and, to a lesser extent, in India and Japan as a source of the insecticidally active pyrethrin ingredients. The increased application of gas chromatographic (GC) techniques to studies of pyrethrum extracts has created renewed interest in research activities with the active components of the extracts, such as studies on the relative toxicity of the pyrethrin I and pyrethrin II fractions to the housefly (*Musca domestica*) and other insects and also the application of modified techniques for the measurement of the relative amounts of these two components in the extracts.

Published reports have been somewhat contradictory in so far as pyrethrin I has been considered to be more toxic than pyrethrin II (and *vice versa*) to the housefly[2–9] and perhaps to other insects. It has been suggested that this anomalous observation may be attributed to poorer storage stability characteristics of pyrethrin II and it has been shown that insecticidal activity is diminished when pyrethrins are exposed to sunlight and air[10].

To date, six insecticidally active esters have been identified in pyrethrin extracts[11–15]: pyrethrin I (Py_I), jasmolin I (J_I), cinerin I (C_I), pyrethrin II (Py_{II}),

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jasmolin II (J_{II}) and cinerin II (C_{II}). Prior to the advent of GC, accepted methods of analysis for pyrethrins defined two insecticidally active fractions, Py_I and Py_{II}, in which each fraction contained Py_I, J_I, C_I, and Py_{II}, J_{II}, and C_{II}, respectively[16,17]. In this paper, the 3-component Py_I group and the 3-component Py_{II} group will be referred to as Py_1 and Py_2, respectively. Results previously reported by other workers were based on Py_1:Py_2 ratios and not on the true component pyrethrin I:pyrethrin II ratios[18]. This paper presents results on the six components of pyrethrin extracts obtained from various world sources utilizing flame ionization GC techniques with variable operational conditions of isothermal, temperature-programmed and matrix-programmed modes, plus mass spectral data obtained by a combined GC–mass spectrometric technique. Results are presented on the "true" Py_I:Py_{II} ratio values and these results are compared with the mixed component Py_1:Py_2 results obtained by previously discussed methods of analysis.

MATERIALS AND METHODS

Gas chromatograph

An F & M Model 810 instrument with a flame ionization detector was used. Three sets of operating conditions were employed, as follows.

(1) Isothermal (at various temperatures, to establish the best conditions), with oven temperatures of 160°, 170°, 180° and 190°, injector temperatures of 180°, 190° and 200° and detector temperatures of 180°, 190° and 210° and a nitrogen flow-rate of 30 or 45 ml/min, a hydrogen flow-rate of 25 ml/min and an air flow-rate of 210 ml/min.

(2) Temperature-programmed, with the oven temperature starting at 155°, and increasing at the rate of 4°/min up to 210° and maintained at that temperature.

(3) Matrix-programmed. The initial oven temperature was 155° and as soon as the solvent peak appeared, the temperature was maintained for 6 min 2 sec (timed with a stop-watch). The temperature was then raised to 175° at the rate of 20°/min. When the Py_I peak reached the maximum height on the recorder chart, the temperature was maintained at 175° for 2.25 min; the temperature was then raised to 205° at the rate of 20°/min and maintained at this temperature until the Py_{II} peak and other subsequent peaks appeared on the recorder chart. The other operating conditions for the gas chromatograph were an injector port temperature of 190° and a detector temperature of 210°.

Gas chromatograph–mass spectrometer

A Finnigan Model 3000 peak identifier was used with a sensitivity of 10^{-6} A/V, electron multiplier high voltage —2.00 kV and electron energy —69.5 V; a mass spectrogram was taken at the apex of each peak. The column was 18 in. ×2 mm I.D., 2.5% XE-60 on Chromosorb W, 60–100 mesh, the helium flow-rate was 20 ml/min and the system was matrix-programmed as described above.

Gas chromatographic column

Thirty different column material preparations were examined in an effort to find a column with optimum conditions for the separation of the six pyrethrin esters on the gas chromatograph (Table I). A column mixture which satisfied these

TABLE I
GC COLUMN MATERIALS USED IN STUDY OF SIX PYRETHRIN ESTERS

Stationary phase	*Support**	*Column*			
		*Type***	*Dimensions*		*Usefulness****
			Length (ft.)	*O.D. (in.)*	
Carbowax 20M, 3%	Chromosorb W, 60–80 mesh	G	4	1/4	U
DC 200, 5%	Chromosorb W, 60–80 mesh	G	4	1/4	U
FFAP, 5%	Chromosorb W, 60–80 mesh	G	4	1/4	U
Neopentyl glycol succinate, 1%	Chromosorb W, 60–80 mesh	G	4	1/4	U
Neopentyl glycol succinate, 3%	Chromosorb W, 60–80 mesh	G	4	1/4	U
Neopentyl glycol succinate, 1.5%	Supelcoport, 80–100 mesh	G	4	1/4	U
OV-17, 3%	Chromosorb W, 60–80 mesh	G	4	1/4	U
OV-22, 2%	Chromosorb W, 60–80 mesh	G	4	1/4	U
OV-25, 2%	Chromosorb W, 60–80 mesh	G	4	1/4	U
OV-101, 2%	Chromosorb W, 60–80 mesh	G	4	1/4	U
OV-210, 2%	Chromosorb W, 60–80 mesh	G	4	1/4	U
OV-225, 2%	Chromosorb W, 60–80 mesh	G	2	1/4	U
OV-225, 5%	Chromosorb W, 60–80 mesh	G	4	1/4	U
QF-1, 5%	Anakrom SD, 70–80 mesh	G	4	1/4	U
QF-1, 5%	Chromosorb W, HP, 80–100 mesh	G	4	1/4	U
SE-30, 3%	Chromosorb W, 60–80 mesh	G	2	1/4	U
SE-30, 3%	Chromosorb W, 60–80 mesh	G	4	1/4	U
SE-30, 5%	Chromosorb W, 60–80 mesh	G	2	1/4	U
SE-30, 5%	Chromosorb W, 60–80 mesh	G	4	1/4	U
SE-52, 3%	Chromosorb W, 60–80 mesh	G	4	1/4	U
SF-96, 2%	Chromosorb W, 60–80 mesh	G	4	1/4	U
XE-60, 2%	Chromosorb W, 60–80 mesh	SS	3	1/8	U
XE-60, 2%	Chromosorb W, 60–80 mesh	G	4	1/4	U
XE-60, 2%	Chromosorb W, 60–80 mesh	SS	5	1/8	U
XE-60, 3%	Chromosorb W, 60–80 mesh	SS	3	1/8	U
XE-60, 3%	Chromosorb W, 60–80 mesh	G	4	1/4	U
XE-60, 3%	Chromosorb W, 60–80 mesh	SS	5	1/8	U
XE-60, 3%	Chromosorb W, HP, 80–100 mesh	G	4	1/4	U
XE-60, 2.5%§	Chromosorb W, 60–100 mesh	G	2	1/4	S
XF-1105, 2%	Chromosorb W, 60–80 mesh	G	4	1/4	U

* All supports are AW-DMCS.
** G = glass; SS = stainless steel.
*** S = satisfactory; U = unsatisfactory.
§ A mixture of equal parts of 2% XE-60 on Chromosorb W, 60–80 mesh, and 3% XE-60 on Chromosorb W, 80–100 mesh.

conditions consisted of 2.5% XE-60 on Chromosorb W AW-DMCS, 60–100 mesh, packed in a 2 ft. × 1/4 in. I.D. glass column. This preferred column material was prepared by mixing equal parts of (1) 2% XE-60 on Chromosorb W AW-DMCS, 60–80 mesh, and (2) 3% XE-60 on Chromosorb W AW-DMCS, 80–100 mesh. Matrix-programmed conditions for the gas chromatograph gave the most satisfactory resolution of the six major pyrethrin esters.

Pyrethrin extracts

Refined and crude pyrethrin extracts from Ecuador, Kenya and Tanzania were supplied by Mr. Dean Kassera, McLaughlin Gormley King Co., Minneapolis, Minn., U.S.A. Extracts from areas of Japan were supplied by Mr. Takenosuke Takano, Institute for Japanese Pyrethrum Research, Kyoto, Japan. Extracts from the area of Srinagar, Kashmir, India, were supplied by Dr. S. Prasad, Council of Scientific and Industrial Research, Jammu-Tawi, India. World Standard extracts for the years 1970 and 1972 were supplied by Dr. S. W. Head, the Pyrethrum Bureau, Nakura, Kenya.

Preparation of sample extracts and World Standard solutions for analysis

A 0.4-g sample of the World Standard (21.3% purity) was weighed into a 10-ml calibrated flask and made up to volume with redistilled carbon disulphide. The standard solution was stored in a brown bottle. Samples of the pyrethrin extracts (about 100 mg per 10 ml as pyrethrins) were weighed into 10-ml calibrated flasks and made up to volume with redistilled carbon disulphide. Then 2–3-μl aliquots of the solutions were applied to the gas chromatograph for analysis. The crude extracts were passed through Florisil columns prior to analysis, as previously described[19].

TABLE II

Py_1:Py_2 AND Py_I:Py_{II} RATIOS OF PYRETHRINS FROM VARIOUS WORLD SOURCES

Pyrethrin extract	Py_1:Py_2* (*AOAC*)**	Py_I:Py_{II}* (*GC method****)
World Standard, 1970	1.12	1.63
World Standard, 1970 (restandardized 2 years later)	1.13	1.40
World Standard, 1972	1.13	1.63
Ecuador, crude	1.54	2.01
Ecuador, crude, cleaned up§	—	2.18
Ecudor, refined	1.53	2.05
India, crude	—	2.10
Japan, Fumakira	0.77	1.22
Japan, Nagaoka	1.08	1.58
Japan, Dainihon Jyochukiku	1.72	0.00
Kenya, crude	0.96	1.49
Kenya, crude, cleaned up§	—	1.46
Kenya, refined	0.94	1.36
Tanzania, crude	1.01	1.42
Tanzania, crude, cleaned up§	—	1.53
Tanzania, refined	1.08	1.47

* $Py_1:Py_2=(Py_I+J_I+C_I)/(Py_{II}+J_{II}+C_{II})$.

** The values are based on the results obtained by the method of the Association of Official Agricultural Chemists (AOAC), U.S.A.[20], and were supplied by the donors of the samples.

*** The values are based on data obtained from a gas chromatograph containing a 2 ft. × 1/4 in. glass column packed with 2.5% XE-60 on Chromosorb W, 60–100 mesh (see text). Each value is based on the average of 3 or more determinations.

§ Florisil column clean-up.

Quantitative measurement of pyrethrin esters

The areas of the pyrethrin ester peaks recorded on the gas chromatograph chart were determined with a planimeter. The World Standard peaks were used as the frame of reference; 20 mg of the World Standard was arbitrarily selected and all other sample peaks were recalculated to this sample size for ready comparison. Py_I: Py_{II} ratios were determined and are presented in Table II.

RESULTS AND DISCUSSION

As previously discussed elsewhere[19,21], the classical AOAC analytical procedure for the determination of "pyrethrins"[20] involves the hydrolysis of the samples and subsequent measurement of the chrysanthemum monocarboxylic and dicarboxylic acids. Thus, the pyrethrin 1 value would encompass the total value for pyrethrin I, jasmolin I and cinerin I; similarly, a single value would be obtained for the pyrethrin 2 fraction. Such values would also include any other potentially hydrolyzable esters, thereby inflating the true value; hydrolyzed degraded esters would also be measured. Obviously, such results could possibly present difficulties

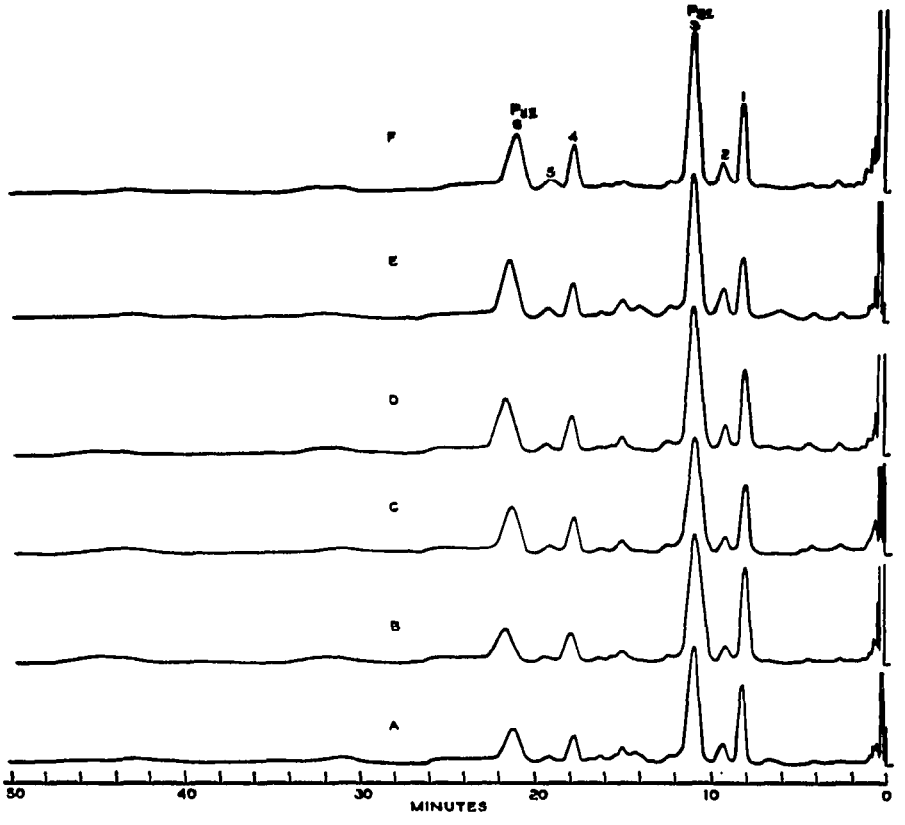


Fig. 1. GC curves of pyrethrin extracts. (A) Ecuador crude; (B) Ecuador refined; (C) Tanzania crude; (D) Tanzania refined; (E) Kenya refined; (F) World Standard, 1970. Peaks are (1) cinerin I; (2) jasmolin I; (3) pyrethrin I; (4) cinerin II; (5) jasmolin II; (6) pyrethrin II.

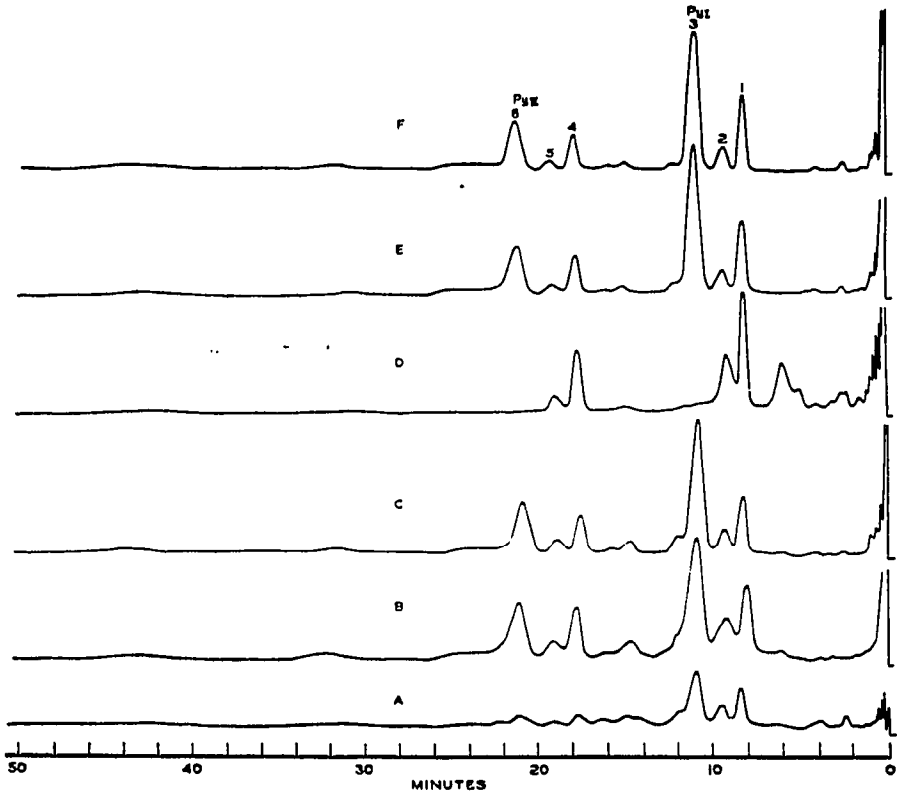
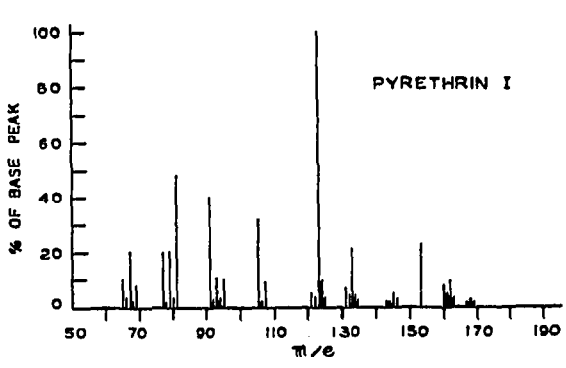


Fig. 2. GC curves of pyrethrin extracts. (A) India crude; (B) Fumakira, Japan, refined; (C) Nagaoka, Japan, refined; (D) Dainihon, Japan, refined; (E) World Standard, 1972; (F) World Standard, 1970. Peaks are (1) cinerin I; (2) jasmolin I; (3) pyrethrin I; (4) cinerin II; (5) jasmolin II; (6) pyrethrin II.

in any attempt to relate the insecticidally active ingredients of the pyrethrin extract to its true toxicity capabilities.

The results given in Figs. 1 and 2 and Table II, and verified by the mass spectral data shown in Figs. 3, 4 and 5[22,23] and Table III, illustrate that GC analyses of pyrethrin mixtures, under the conditions prescribed above, will provide the analyst and the insect toxicologist with information useful for a more precise evaluation of the insecticidal properties of a given pyrethrin extract. For example, the AOAC procedure for the Japanese samples (Table II) produced positive values for the pyrethrin 1 and 2 components. However, the GC procedure showed that one of the Japanese samples did not contain any measurable amounts of pyrethrin I and pyrethrin II (see Fig. 2D); subsequent investigation revealed that the sample was 10 years old, which (storage conditions unknown) suggested that complete degradation of the two components had occurred over this extended period of time. Again referring to Table II, the Py_I: Py_{II} ratios for the Equador and Indian samples were the same, yet a comparison of the gas chromatograms (Fig. 1A and Fig. 2A) indicated that on an equivalent weight basis the Ecuador extracts were superior in quality.



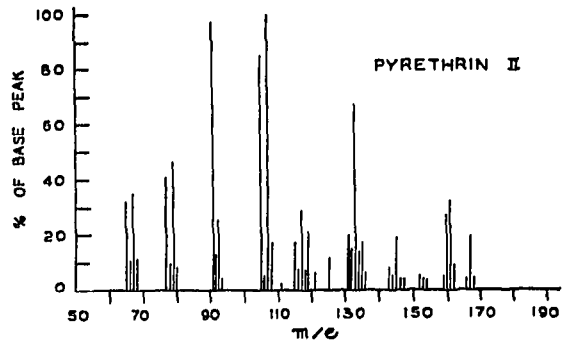
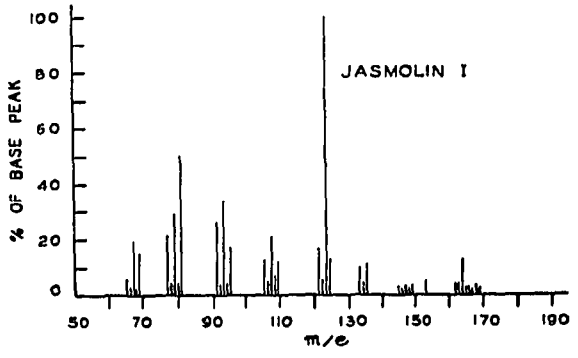


Fig. 3. Mass spectra of pyrethrins I and II.



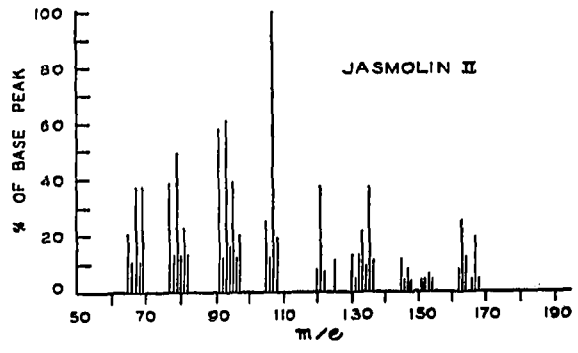
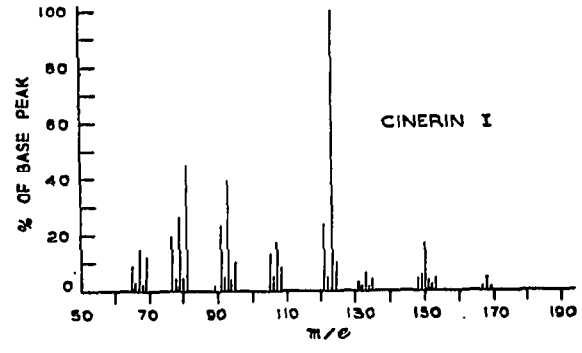


Fig. 4. Mass spectra of jasmolins I and II.



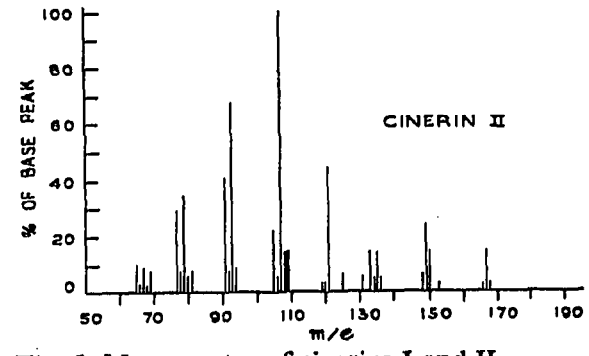


Fig. 5. Mass spectra of cinerins I and II.

Results obtained by the GC procedure are considerably more informative than those obtained by the AOAC procedure. For example, with the exception of the erratic results obtained in the Japanese samples, the African extracts contained approximately equal amounts of the Py_1 and Py_2 component mixtures and the Ecuador extracts contained about 1.5 times as much Py_1 components compared with their Py_2 content (Table II). However, when comparing the amounts of the two predominant insecticidally active components, Py_I and Py_{II}, obtained by GC, the amount of true Py_I was about 1.5 times the amount of Py_{II}. The exceptions were the Ecuador and Indian samples, which contained twice as much Py_I when compared tc their Py_{II} content.

The mass spectral data (Table III) indicate that the fragmentation patterns

TABLE III

INTENSITIES OF FRAGMENT IONS IN THE MASS SPECTRA OF THE PYRETHRIN I AND PYRETHRIN II ESTERS

Values are expressed as a percentage of the base peak.

m/e	*Pyi*	*Ji*	*Ci*	*Pyii*	*Jii*	*Cii*
168	3.0	3.3	2.8			
167				18.4	18.4	13.7
164		13.0			11.4	
163		3.5			24.6	
162	10.0	4.0		8.6	8.0	
161	3.5			32.0		
160	7.7			27.0		
153	2.3	3.5	2.2	3.4	6.0	1.7
150			17.0			13.7
149			3.9			23.0
148			3.2			6.0
147					8.0	
145	5.0	2.6		15.3	10.5	
143	2.0			8.6		
136				6.1	10.5	4.0
135	1.5	11.0			37.0	13.3
133	21.0	9.6	4.5	67.0	21.0	14.0
131			2.1			4.4
125	2.7			10.4	10.5	5.6
123	100.0	100.0	100.0			
121		16.5	22.6	6.1	37.7	44.0
117				29.0		
111				1.0		
108	4.8	7.8	7.3	17.0		13.7
107	8.6	20.8	16.7	100.0	100.0	100.0
105	31.5	13.0	12.2	85.0		21.0
94	2.3			3.0	15.0	8.0
93		33.5	38.4		61.0	67.0
91	40.0	26.0	23.0	97.0	58.0	40.7
81	48.0	50.0	42.7		22.0	
80				7.4	12.2	4.8
79		28.7	24.4		49.0	34.0
77		20.4	19.0		38.0	29.0
67	20.0	18.7	14.0	35.0	37.0	9.0
66	4.0			10.0		3.0

of the six pyrethrin esters are similar to those previously reported by King and Paisley[22] and Pattenden *et al.*[23]. The relative intensities of the major fragments are variable, especially for the pyrethrin II esters. No molecular ions were observed for any of the esters and no fragment ions were observed above *m/e* 168 for pyrethrin I esters and *m/e* 167 for pyrethrin II esters. The pyrethrin 1 esters (Py_I, J_I and C_I) had base peaks at *m/e* 123 and similar fragment ions at *m/e* 168, similar to previously published results[22,23]. The pyrethrin 2 esters (Py_{II}, J_{II} and C_{II}) had base peaks at *m/e* 107, in contrast to an *m/e* 133 peak for pyrethrin II, *m/e* 163 for jasmolin II, and *m/e* 149 for cinerin II reported earlier[22,23]. However, the fragment ions *m/e* 133, 163 and 149 had greater intensities for the pyrethrin II esters than for the pyrethrin I esters. All of the pyrethrin II esters revealed clusters of three ions separated by one mass unit; *m/e* 160, 161 and 162 for pyrethrin II; *m/e* 162, 163 and 164 for jasmolin II; and *m/e* 148, 149 and 150 for cinerin II. Similar results were reported by Pattenden *et al.*[23]. These clusters were also evident in mass spectra of the pyrethrin I esters but were of lower intensity for the respective clusters.

The differences in intensities of the fragmentations observed with the Finnigan peak identifier may be attributed to the higher temperatures applied to the pyrethrin 1 and 2 mixtures. The temperature of the gas chromatograph column was first set at 175° and maintained at this temperature until the three esters of the pyrethrin I group were eluted; then the temperature was raised to 205° to effect the elution of the three esters of the pyrethrin II group. The eluate from the GC column was then passed through a heated gas chromatograph–mass spectrometer interface set at 225° and finally through a heated transfer line at a temperature of 250°. The electron voltage was set at 69.5 V with no tangible differences in fragment ion intensity within a range from 30 to 100 V[24].

The acquisition of analytical data on pyrethrum as discussed in this paper should prove informative and helpful in the selection of a pyrethrin mixture for subsequent insecticidal use and it should aid the insect toxicologist in his efforts to interpret the toxic effects of pyrethrin mixtures on insect life.

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