ACTION OF HEAT ON PYRETHRUM EXTRACT: THE ISOMERISATION OF PYRETHRINS TO ISOPYRETHRINS

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The thermal isomerisation of the pyrethrins to isopyrethrins has been studied, the progress of the reaction being followed by the rise in optical density at 2700 Å and the fall at 2300 Å. The isomerisation is a first-order reaction with 10^6k equal to 4·58 and 481 at 125° and 195° respectively; these values give $E^* = 24{,}610$ cal. mol.$^{-1}$; log $A = 8{\cdot}17$; $\Delta S^* = -21{\cdot}8$ cal. deg.$^{-1}$.

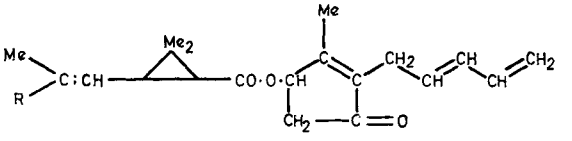
In normal (unheated) pyrethrum extract the ratio of the optical densities at 2700 Å and 2300 Å is 0·08; in pyrethrum extract which has been fully isomerised by heat this ratio is 0·62.

The biological activities of normal and of partially and completely isomerised pyrethrum extract have been examined by three methods (two on flies and one on grain weevils). Extract in which the pyrethrins have been completely isomerised to isopyrethrins has approximately one-half of the lethal, and one-quarter of the knockdown, activity of normal extract on houseflies.

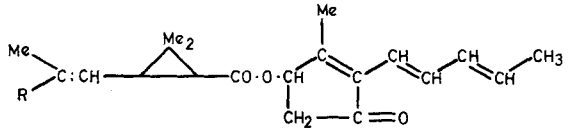
Pyrethrum extract which has been submitted to heat treatment shows no loss in biological activity if the ratio of the optical density at 2700 Å to that at 2300 Å has not risen above 0·095.

Introduction

The structural alteration of the pyrethrins effected by heat was first observed by Staudinger & Ruczicka,[1] and confirmed by Haller & LaForge[2] who reported that the biological activity of pyrethrum extract decreased when the material was subjected to prolonged heating. Brown *et al.*[3] submitted pyrethrum extract which had been heated to 200° for ½ h. to displacement chromatography and separated, in addition to the four known original constituents, two new compounds which they termed isopyrethrins I and II for which structures c and d were advanced from spectrophotometric evidence.



(*a*, Pyrethrin I, R = Me)
(*b*, Pyrethrin II, R = CO_2Me)



(*c*, Isopyrethrin I, R = Me)
(*d*, Isopyrethrin II, R = CO_2Me)

The new isomers are formed by a shift of the double bonds of the pentadienyl side-chain into conjugation with the double bond of the cyclopentenolone ring. It was assumed that cinerin I and cinerin II are unchanged by the heat treatment. Elliott *et al.*[4] confirmed this structure and reported that isopyrethrin I was only one-sixteenth as toxic to mustard beetles (*Phoedon cochleariae*) as pyrethrin I.

The thermal isomerisation of the pyrethrins to the isopyrethrins is considered to be a free-radical reaction in which the rate-determining process is the removal of a hydrogen atom from the linolenic methylene group. In the free radicals the π-electrons in the side-chain become mobile and will find the lowest energy level; this will be the assembly of maximum conjugation, viz., the isopyrethrin structure.

The present work was carried out in order to obtain information on the rate of transformation of the pyrethrins to isopyrethrins at different temperatures. Such information is

of value in the problems encountered in distilling pyrethrum oleoresin in order to produce a virtually colourless extract without loss of biological activity.

Experimental and results

A solution of pyrethrum extract in kerosene, sealed in ampoules, was heated in a bath of refluxing amyl alcohol or kerosene at 125° and 195° respectively (atmospheric pressure at Nakuru 620 mm. Hg). After a given time an ampoule was removed, opened, the contents analysed and the ultra-violet absorption spectrum in ethanol solution plotted from 2100 to 2900 Å.

Table I (Fig. 1) and Table IA (Fig. 2) show the effect of heating pyrethrum extract at 125° and 195° respectively; Tables II and IIA and Tables III and IIIA are the results obtained with substantially pure pyrethrin I/cinerin I and pyrethrin II/cinerin II prepared[5] by fractional distillation of pyrethrum oleoresin in a 2-in. wiped-wall molecular still. The Tables show that as the heating continues there is a progressive fall in optical density at 2300 Å and rise at 2700 Å.

Table I

Analyses of pyrethrum extract heated at 125°

Heating time, h.	DNP analysis* Py I, %	DNP analysis* Py II, %	DNP analysis* Total Py, %	PBK analysis Py I, %	PBK analysis Py II, %	PBK analysis Total Py, %	Spectroscopic analysis (total Py)	Ratio d_{2700}/d_{2300}	% Isomerised
0	4·67	2·91	7·58	5·74	3·58	9·32	9·78	0·081	0
8	4·56	2·84	7·40	5·68	3·16	8·84	9·52	0·125	14
21	4·49	2·80	7·29	5·44	3·22	8·66	9·22	0·188	31
48	4·44	2·47	6·91	5·01	2·02	7·03	8·34	0·294	54
74	4·40	2·31	6·71	4·55	2·06	6·61	7·60	0·348	64
96	4·08	2·25	6·33	4·21	1·92	6·13	7·20	0·424	76

Table IA

Analyses of pyrethrum extract heated at 195°

0	5·37	3·39	8·76	7·08	3·90	10·98	11·0	0·081	0
¼	5·03	3·02	8·05	7·01	3·81	10·82	9·5	0·196	33
½	4·44	2·51	6·95	6·60	3·87	10·47	7·9	0·326	60
1	3·82	2·01	5·83	6·41	3·74	10·15	6·6	0·489	85
2	3·74	1·85	5·59	6·00	3·58	9·58	6·5	0·579	96
2½	3·97	1·91	5·88	5·81	3·55	9·36	6·1	0·620	100
3	3·82	1·79	5·61	5·24	2·96	8·20	6·0	0·630	100

d_{2700}/d_{2300} is ratio of optical densities at 2700 and 2300 Å Py = Pyrethrin

* A pyrethrum extract analysing at 25·0% pyrethrins by the PBK method (Official Method of the Pyrethrum Board of Kenya, September, 1954) analyses at 22·3% pyrethrins by the A.O.A.C. method ('Official Methods of Analysis of the Association of Official Agricultural Chemists', 1950, 7th Edn) and 20·0% pyrethrins by the DNP method (Smith[6a]). For this reason the method of assay should be quoted when stating specific extinction coefficients. The spectrophotometric analyses in all the tables is obtained by using $E_{1\,cm.}^{1\%\ PBK} = 900$ (at 2300 Å). (It should be noticed that it is normal practice for the Pyrethrum Board of Kenya to determine optical densities at 2300 instead of the usual 2270 Å.)

In normal unheated pyrethrum extract $E_{1\,cm.}^{1\%\ PBK*} = 900$ (at 2300 Å) and 72 (at 2700 Å), the ratio of the optical density at 2700 Å to that at 2300 Å being 0·08. In pyrethrum extract which has been heated until this ratio attains its maximum value, viz., 0·62 after 2½ h. at 195°, $E_{1\,cm.}^{1\%\ PBK} = 500$ (at 2300 Å) and 310 (at 2700 Å); it is assumed that *all* the pyrethrins initially present have isomerised to isopyrethrins (see footnote to Table IA). Accordingly in any heat-treated extract in which x is the proportion of the initial pyrethrins which have isomerised to isopyrethrins it follows that

$$r = \frac{d_{2700}}{d_{2300}} = \frac{310x + 72(1 - x)}{500x + 900(1 - x)}$$

Hence

$$x = \frac{900r - 72}{400r + 238}$$

This gives the extent of the isomerisation in terms of the d_{2700}/d_{2300} ratio.

* 1% solution of pyrethrins as determined by the PBK method

Table II

Analyses of pyrethrin I/cinerin I heated at 125°

Heating time, h.	DNP analysis Py I, %	Py II, %	Total Py, %	Ratio d_{2700}/d_{2300}
0	4·76	0·44	5·20	0·070
20	4·20	0·39	4·59	0·125
48	4·18	0·40	4·58	0·198
75	4·01	0·40	4·41	0·248
92	3·87	0·40	4·27	0·280

Table IIA

Pyrethrin I/cinerin I heated at 195°

Heating time, h.	DNP analysis Py I, %	Py II, %	Total Py, %	Ratio d_{2700}/d_{2300}
0	4·76	0·44	5·20	0·070
½	4·34	0·41	4·75	0·193
1	3·88	0·39	4·27	0·361
2	3·44	0·39	3·83	0·512
4	3·09	0·40	3·49	0·518

Table III

Analyses of pyrethrin II/cinerin II heated at 125°

Heating time, h.	DNP analysis Py I, %	Py II, %	Total Py, %	Ratio d_{2700}/d_{2300}
0	0·31	2·33	2·64	0·100
8	0·37	2·06	2·43	0·156
23	0·42	1·86	2·28	0·218
49	0·50	1·71	2·23	0·288
72	0·52	1·69	2·21	0·367
96	0·54	1·34	1·88	0·433

Table IIIA

Pyrethrin II/cinerin II heated at 195°

Heating time, h.	DNP analysis Py I, %	Py II, %	Total Py, %	Ratio d_{2700}/d_{2300}
0	0·31	2·33	2·64	0·100
¼	0·41	2·01	2·42	0·218
½	0·49	1·53	2·02	0·382
1	0·60	1·15	1·75	0·578
3	0·59	0·77	1·36	0·634
4	0·61	0·72	1·33	0·640

In the six families of ultra-violet absorption curves relating to Tables I–III all the component curves intersect at 2440 Å (see Figs. 1 and 2). The specific extinction coefficient at this wavelength therefore has the same value irrespective of whether the pyrethrins have undergone partial or complete isomerisation to isopyrethrins. From this it follows that, for an extract which has or has not been damaged by heat: $E^{1\%\ PBK}_{1\ cm.}$ at 2440 Å = 430.

For the isomerisation of pyrethrum extract the proportion of the initial pyrethrins converted to isopyrethrins has been calculated from the optical densities at 2700 Å and entered in Tables I and IA. The first-order reaction equation $kt = \ln \alpha(\alpha - x)$, where α is the initial concentration of pyrethrins and x the concentration of isopyrethrins at time t, to give 10^6k, for reaction times 8, 24, 48, 72 and 92 h. at 125° respectively, equal to 5·2, 4·9, 4·5, 4·0 and 4·3 (average 4·58 sec.$^{-1}$). At 195° the values for 10^6k, for reaction times ¼, ½, 1 and 2 h., are, respectively, 443, 508, 526 and 446 (average 481 sec.$^{-1}$). If the Arrhenius and Eyring equations

$$k = A \exp(-E^*/RT) \quad \text{and} \quad k = (RT/Nh) \exp(-E^*/RT).\exp(\Delta S^*/R)$$

where $R = 1{\cdot}987$ cal. deg.$^{-1}$, R/N (Boltzmann constant) = $1{\cdot}3805 \times 10^{-16}$ erg deg.$^{-1}$, h (Planck constant) = $6{\cdot}624 \times 10^{-27}$ erg. sec.$^{-1}$, are applied to these values for k, it follows that $E^* = 24{,}610$ cal mol.$^{-1}$, $\log A = 8{\cdot}17$; and $\Delta S^* = -21{\cdot}8$ cal. deg.$^{-1}$. The negative ΔS^* reflects the loss in rotational freedom about the bond between the 2-position in the cyclopentenolone ring and the 1′-position of the pentadienyl side-chain when the transition structure is formed. In the mesomeric transition structure this bond must assume partial double-bond character with consequent loss of rotational and flexing freedom of the whole side-chain with respect to the plane of the cyclopentenolone ring.

In another series of experiments, 20% pyrethrum extract containing 6% of ethyl alcohol (a wax co-solvent) was diluted with an equal volume of xylene and this solution vigorously refluxed under a double surface condenser. The vapours above the boiling liquid protected the pyrethrins from atmospheric oxygen. The internal temperature, initially 122°, slowly rose to 128°/620 mm., the solution doubtless losing ethyl alcohol slowly through the condenser. The solution was sampled at intervals for analysis. It was observed in these experiments that there was considerable time lag before the optical density at 2300 Å began to fall and that at 2700 Å began to rise. Eventually, after heating for 100 h., the ratio of optical densities at 2700 Å and 2300 Å rose to its normal maximum of 0·62. Table IV is typical of a number of such sets of experiments; all the ultra-violet absorption curves intersect at 2440 Å. Although the evidence is not conclusive, it would appear that the ethyl alcohol initially present inhibits the isomerisation, since the same formulation which did not contain alcohol showed the normal

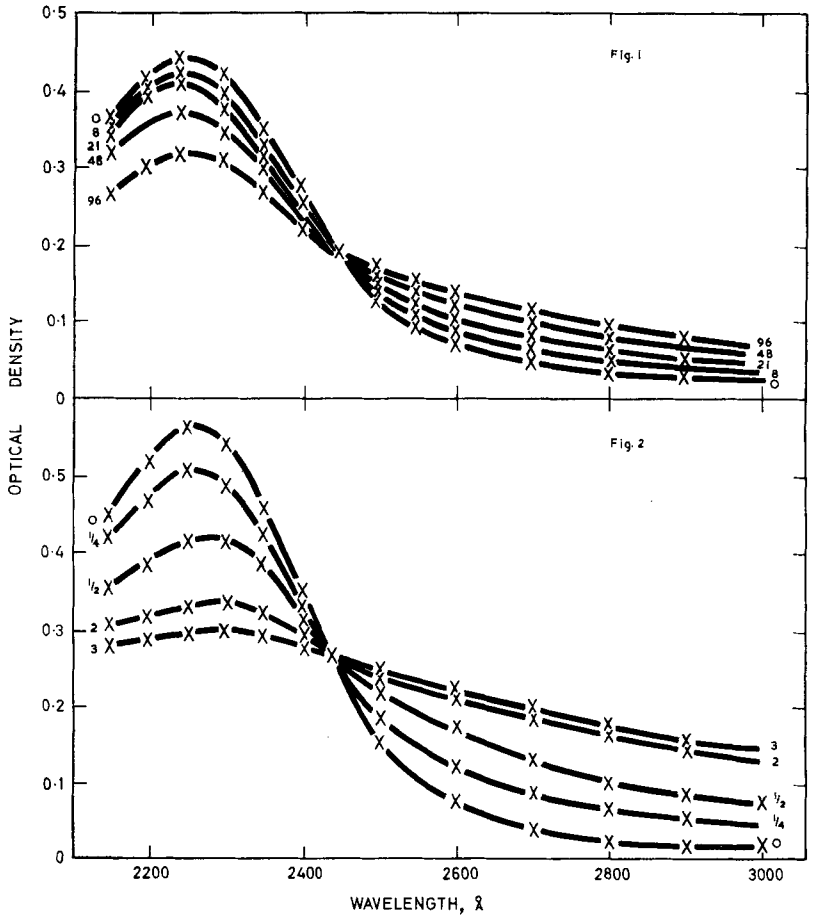


FIG. 1.—*Spectrum of pyrethrum extract* (9·32% *pyrethrins PBK*) *heated for* 0, 8, 21, 48 *and* 96 *h. at* 125°
(50 mg. of extract per l. in ethyl alcohol; 1 cm. cell)

FIG. 2.—*Spectrum of pyrethrum extract* (10·98% *pyrethrins PBK*) *heated for* 0, ¼, ½, 2 *and* 3 *h. at* 195°
(60 mg. of extract per l. in ethyl alcohol; 1 cm. cell)

progressive rise in the ratio of the optical densities at 2700 Å and 2300 Å (Table V). (Compare the protective influence of piperonyl butoxide[7] on the photolysis of the pyrethrins.)

Effect of heat on the chemical components of pyrethrum extracts

It can be seen from Table I that the total pyrethrins (PBK method) in a solution of pyrethrum extract in kerosene fall from 9·32% to 8·66% (a fall of 7% of the initial value) when the extract is heated for 8 h. at 125°; the d_{2700}/d_{2300} ratio rises from 0·081 to 0·125. In contrast to this, heating at 125° for 8 h. in a solvent consisting of 48·5% of kerosene, 48·5% of xylene and 3% of ethyl alcohol (Table IV) causes only an insignificant fall in total pyrethrins (PBK method) and an insignificant rise in the d_{2700}/d_{2300} ratio.

At 195° there is a severe pyrolysis and loss of pyrethrins; after 3 h. at this temperature the PBK- and DNP-pyrethrins are only 75% and 64% of their respective initial values. The figures for the DNP analyses indicate that the products of the pyrolysis of pyrethrin II travel down the chromatographic column with the pyrethrin I band; a similar observation has been made after partial hydrolysis[8] of pyrethrum extract with dilute acid.

It was of interest to determine whether there is any loss in pyrethrins, as measured by the PBK method, when pyrethrum oleoresin is submitted for short periods to temperatures of the order of 70–100°. (During the final stages of manufacture, oleoresin is normally heated in

Table IV

Pyrethrum extract containing 10% *pyrethrins,* 50% *xylene and* 3% *ethyl alcohol refluxed at* 122–128°

Time, h.	Method of analysis							Ratio d_{2700}/d_{2309}
	DNP			PBK			Spectroscopic total Py, %	
	Py I, %	Py II, %	Total Py, %	Py I, %	Py II, %	Total Py, %		
0	4·87	3·57	8·44	6·21	4·43	10·64	10·9	0·088
2	4·67	3·59	8·26	6·27	4·42	10·69	10·9	0·083
4	4·64	3·55	8·19	6·29	4·09	10·38	10·8	0·082
6	4·52	3·45	8·07	6·61	4·27	10·80	10·9	0·082
10	4·69	3·35	8·04	6·19	4·22	10·41	10·9	0·093
16	4·42	3·27	7·69	5·98	4·20	10·18	10·1	0·155
22	4·26	2·92	7·18	6·32	4·09	10·41	9·5	0·243
28	4·04	2·92	6·94	6·06	4·33	10·39	8·3	0·351
75	4·15	2·54	6·69	5·78	4·09	9·87	8·1	0·450
100	3·48	2·18	5·66	5·72	4·01	9·73	7·5	0·590

Table V

Pyrethrum extract containing 10% *pyrethrum,* 50% *xylene (no alcohol) refluxed at* 128°

0	4·86	3·42	8·28	6·47	3·99	10·46	11·0	0·088
4	4·49	3·03	7·52	6·28	3·84	10·12	10·1	0·165
8	4·48	2·75	7·23	6·19	3·91	10·10	9·4	0·186
16	4·44	2·64	7·08	6·06	3·86	9·92	8·9	0·400

the falling-film evaporator to ~75°/25 mm. Hg for a few seconds in order to remove traces of isohexane.) Accordingly, a sample of 25% oleoresin was sealed in twelve 5-c.c. ampoules. Six of these were heated in an oven at 105° for 1½ h., cooled, opened and analysed; the other six were analysed without any heat treatment. Each analysis was made in duplicate and the average of the duplicates recorded in Table VI.

For the observed mean difference (MD) the statistical parameters are: variance 0·20; standard deviation 0·45; standard error 0·183; 95% confidence limits −0·69 to +0·25; 99% confidence limits −0·96 to +0·52. There is therefore no statistically significant difference between the means of the analyses of the heated and unheated extract and it must be concluded that there is no loss of PBK pyrethrins when oleoresin is stripped of residual solvent at 80–100°/25 mm. in a falling-film evaporator during the final stages of manufacture.

The stability of chrysanthemic acid to heating

The only change in the pyrethrin molecule, other than isomerisation to isopyrethrin, which might occur on heating is inversion of optical configuration at one or more of the three asymmetric centres. LaForge & Green[9] reported the toxicity of (−)*cis*-cineronyl (+)*trans*-chrysanthemate to flies (determined by the Campbell turntable method) to be 1·8 times that of the natural (+)*cis*-cineronyl (+)*trans*-chrysanthemate; Gersdorff[10] found no difference in the activities of (+)*cis*-pyrethronyl (+)*trans*-chrysanthemate and (*rac.*)*cis*-pyrethronyl (+)*trans*-chrysanthemate. It must be accepted upon this evidence that inversion at C_4 of the cyclopentenolone ring would either not effect or would cause a slight increase in the biological activity.

Table VI

Effect of heating oleoresin at 105° *for* 1½ *h. on the PBK analysis*

% Pyrethrins			Difference X	(X − MD)	(X − MD)²
Unheated	Heated				
26·58	25·97		−0·61	-39×10^{-2}	1521×10^{-4}
26·45	25·83		−0·62	−40	1600
25·71	25·74		+0·03	+25	625
25·79	26·07		+0·28	+50	2500
26·16	25·52		−0·64	−42	1764
25·11	25·32		+0·21	+45	2025
Mean 25·97	Mean 25·75	Mean (MD)	−0·22		S, 10035×10^{-4}

Inversion at C_1 and C_3 of the cyclopropane ring, however, would substantially reduce toxicity since the relative activities of a given pyrethrolone esterified with (+)*trans*-, (+)*cis*-, (−)*trans*- and (−)*cis*-chrysanthemic[11] acids are (+)*trans* > (+)*cis* ⩾ (−)*trans* > (−)*cis*. It was therefore important to ascertain the stability of (the natural) (+)*trans*-chrysanthemic acid when subjected to temperatures of the order of 200–220°.

A sample of (+)*trans*-chrysanthemic acid (Found $[\alpha]_D^{20}$ + 14·32° (ethanol) ; lit.[12] + 14·2°) was heated from 25° to 200° during 1 min., held at 215–225° for 2 min., and cooled to room temperature during 1 min. The specific rotation of the products was then $[\alpha]_D^{20}$ + 14·31° (ethanol) showing that the configurational integrity of the molecule had not been affected by the heat treatment. Chrysanthemic acid obtained by hydrolysis of oleoresin which had been distilled at 230°/5 μ in a 12-in. industrial molecular still[5] had b.p. 114–116°/1 mm. and $[\alpha]_D^{20}$ + 14·31°.

The biological activity of heat-treated pyrethrum extract

Various heat-treated pyrethrum extracts together with their unheated controls were subjected to bioassay in order to determine the extent of any impairment of biological activity due to heat treatment and to relate the loss in activity to the d_{2700}/d_{2300} ratio.

The standard techniques used were : (i) Kearns & March method[13] for knockdown of flies ; (ii) the measured drop method for kill of flies ;[14] (iii) the dusted wheat method[15] for kill of *Calandria oryzae*. For the preparation of the required dilutions for the bioassays the pyrethrin content of the heated concentrate was taken to be the same as the initial concentration before heating : this enables damage to be directly assessed.

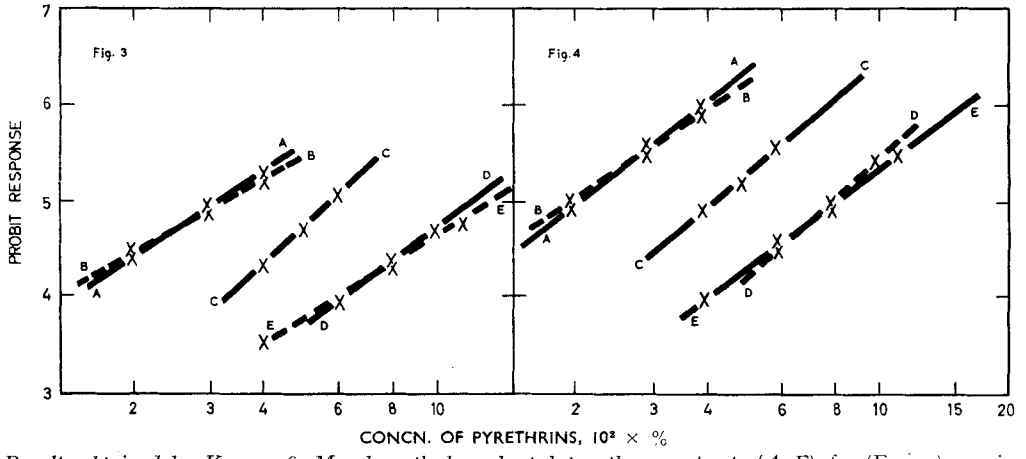
The pyrethrum extracts used were :

(A) unheated control : d_{2700}/d_{2300} = 0·081 ; (B) (A) after being heated 10 h. at 125° in 50% xylene + 3% ethanol solution (d_{2700}/d_{2300} = 0·093) ; (C) (A) after heating 28 h. at 125° in 50% xylene at 3% ethanol solution (d_{2700}/d_{2300} = 0·351) ; (D) (A) after heating 100 h. at 125° in 50% xylene + 3% ethanol solution (d_{2700}/d_{2300} = 0·590) ; (E) (A) after heating 3 h. at 195° in kerosene solution (d_{2700}/d_{2300} = 0·62).

The results obtained are shown in Figs. 3–7 and the KD_{50} and LD_{80} values derived from these figures are given in Tables VII–IX.

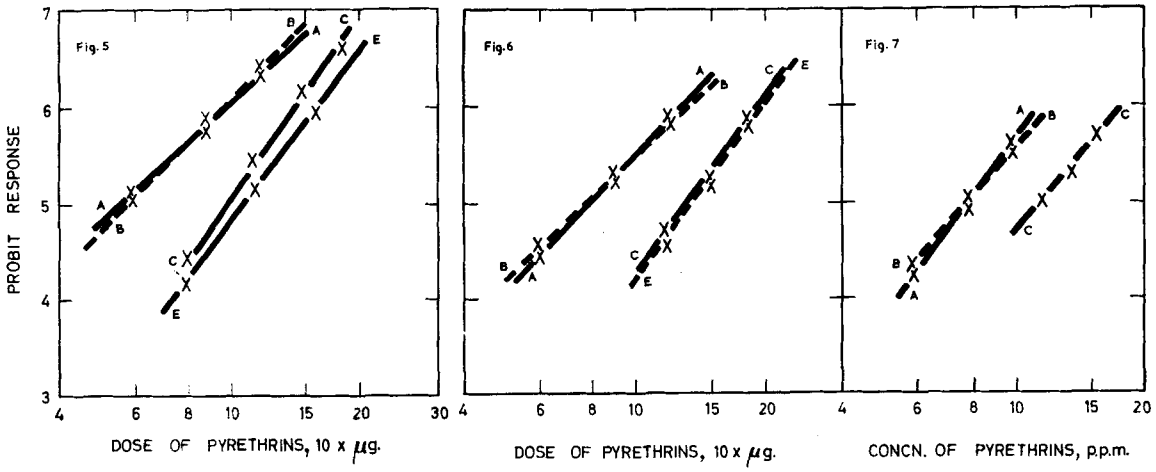
Discussion

The results given above show that the biological activity of pyrethrum extract which has been subjected to heat treatment is a function of the ratio of the optical density of the extract at 2700 Å to that at 2300 Å. This ratio is a measure of the isopyrethrin content of the extract



Results obtained by Kearns & March method on heated pyrethrum extracts (A–E) for (FIG. 3) 5 *min.*, (FIG. 4) 10 *min. knock-down of houseflies*

5-day-old flies ; 80–120 flies per batch. Solutions prepared containing 0·01%–0·12% pyrethrins : 0·2 c.c. sprayed from each nozzle during 5 sec. at 12·5 p.s.i. at 28° : 3 doses : 4–6 replicates on each sample



Results by measured drop technique on heated pyrethrum extracts applied to male (FIG. 5) *and female* (FIG. 6) *houseflies*

5-day-old flies: 30 flies per dose: 4 replicates on each sample. Flies dosed topically with solution of pyrethrins in kercsene and then placed in jars at 27° and supplied with 5% sucrose solution for 24 h.

FIG. 7.—*Results by dusted wheat technique*[15] *on heated pyrethrum extracts (kill of* C. oryzae)

The extracts were mixed with B.P. talc to give dusts containing 1% of pyrethrins (calc. on unheated extract) aud aliquots weighed into and mixed in bottles with 50 g. of wheat. Each bottle was infected with 50 *C. oryzae* spores and kept for 7 days

and, provided this ratio does not exceed a value of 0·095, the heat treatment has caused no damage. This information is of significance in the problems encountered in the distillation of pyrethrum extract and in the design of thermal fogging machines for dispensing pyrethrum mists.

In normal unheated extracts the ratio of the optical densities at 2700 Å and 2300 Å is 0·08, whereas in an extract in which the pyrethrins have been completely isomerised to isopyrethrins the ratio is 0·62 ; such extract has one-half of the lethal, but only one-quarter of the knockdown, activity of normal extract on houseflies. It is of interest that in the mesomeric structure of the isopyrethrins the bond between $C_{(2)}$ of the cyclopentenolone ring and $C_{(1)}$ of the pentadienyl side-chain will have partial double-bond character which will cause substantial loss of rotational and flexing freedom of the whole side-chain with respect to the plane of the cyclopentenolone ring. The remarkably high knockdown activity of the pyrethrins on insects implies a multiplicity of rapidly effected attachments of the pyrethrin molecule to receptor sites in the nerve substrate. This restraint imposed upon the flex and rotation of the side-chain may be the reason for the much greater loss in knockdown activity than in lethal activity when the pyrethrins are converted to isopyrethrins.

It is of interest to consider the thermodynamic aspects of the conversion of pyrethrins to isopyrethrins. The energy and entropy of activation in the process are respectively 24,610 cal. mol.$^{-1}$ and $-21{\cdot}8$ cal. deg.$^{-1}$. Since $\Delta F^* = \Delta H^* - T.\Delta S^*$, the free energy of activation ΔF^* at 225° (the normal temperature for distillation of pyrethrum extract) is 35,460 cal. mol.$^{-1}$. With a free-energy barrier of this magnitude, the transformation cannot take place

Table VII

KD_{50} values (Kearns & March method) derived from Figs. 3 and 4

Extract	d_{2700}/d_{2300} ratio	KD_{50} (5 min.)	KD_{50} (10 min.)	Relative activity
A (unheated control)	0·081	0·032%	0·018%	100
B	0·093	0·031	0·019	100
C	0·350	0·059	0·042	54–43
D	0·590	0·130	0·082	25–21
E	0·620	0·140	0·086	23–21

Table VIII

LD_{50} values derived from the results shown in Figs. 5 and 6

Extract	d_{2700}/d_{2300} ratio	LD_{50} μg./fly male	LD_{50} μg./fly female	Relative potency
A (unheated control)	0·081	0·56	0·80	100
B	0·093	0·58	0·80	100
C	0·350	1·00	1·36	c. 58
E	0·620	1·12	1·40	c. 54

Table IX

LD_{50} values derived from the results shown in Fig. 7

Extract	d_{2700}/d_{2300} ratio	LD_{50} (p.p.m.)	Relative potency
A	0·081	8	100
B	0·093	8	100
C	0·350	12	66

with any high degree of spontaneity. (For example, for the conversion[16] of the comparatively stable diphenyl ether-2-carboxylic acid to xanthone, the energy and entropy of activation are respectively 20,900 cal. mol.$^{-1}$ and -13 cal. deg.$^{-1}$.)

Accordingly it is to be expected that in a wiped-wall falling-film molecular still, in which the pyrethrins are subjected to the high temperature for a very brief period, distillation can be accomplished with no measurable conversion of pyrethrins to isopyrethrins. This has been proved in practice. Elliott *et al.*[17] distilled neat 25% extract in a 2-in. wiped-wall still and found no loss in toxicity to mustard beetles. Goldberg *et al.*[5] have fractionally distilled pyrethrum extract diluted with light liquid paraffin B.P. in the same type of still and have effectively separated pyrethrin I and pyrethrin II. In this connexion it is significant that the pyrethrin II recorded in Table V above had been passed through the still eight times at temperatures ascending from 160 to 220° and the ratio d_{2700}/d_{2300} had not risen above 0·10. It was also shown[5] that it had four times the knockdown activity of pyrethrin I against houseflies and, when synergised 5 : 1 with piperonyl butoxide, twice the knockdown activity of similarly synergised pyrethrin I which had only passed through the still once at 140°. During the last few years pyrethrum extract has been co-distilled[18] with piperonyl butoxide on an industrial scale at temperatures above 200° in falling-film, short-contact stills. Furthermore, in the period 1960–1962 some 200,000 lb. of 25% pyrethrum extract have been co-distilled[5] in Nakuru with light liquid paraffin B.P. in a 12-in. Edwards' molecular still to give a 92·5% yield of decolorised extract all of which had a d_{2700}/d_{2300} ratio of 0·08–0·085, showing it to be free from isopyrethrins. Examination for KD_{50} and LD_{50} against *Musca domestica* and LD_{50} against *Tribolium castaneum* and *C. oryzae* showed[5] no difference between the distilled material, the undistilled oleoresin and nitromethane decolorised extract.

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THE RÔLE OF WHEAT FLOUR PENTOSANS IN BAKING. III.*—Enzymic Degradation of Pentosan Fractions

By PAMELA M. WRENCH

Enzymes in snail digestive juice degrade two of the five fractions obtained when flour pentosans are chromatographed on DEAE cellulose. These fractions are both glycoproteins containing arabinose and galactose and one also contains xylose. Solutions of the latter fraction gel on the addition of oxidising agents, but this ability is lost after incubation with snail juice enzymes. Loaves baked from doughs in which the pentosans have been degraded by snail juice enzymes are inferior in volume, but this effect is not observed in the presence of oxidising agents. The effect of oxidising agents, however, does not appear to be directly related to that of the pentosans.

Introduction

The possible importance of the pentosans of wheat flour in contributing to the rheological and baking properties of a dough has been reviewed in the first paper of this series.[1] Tracey[1] used the technique of enzymic degradation of dough components *in situ* to establish that pentosans play a significant rôle in determining dough properties. Cawley[2] later described the rheological and baking behaviour of gluten–starch doughs reconstituted by the addition of native flour pentosan† or one of a range of polysaccharides of known composition. The present paper describes the fractionation of pentosans extracted from flour and the further use of enzymes to identify the components responsible for their effects in dough. The relation between the formation of gels by pentosans when oxidised to the effect of oxidising agents on doughs has also been studied briefly.

Experimental

Flour

A typical Australian baker's flour (protein 11·9% on a 14% moisture basis) was used throughout.

Pentosan preparations

Flour solubles prepared as described by Cawley[2] were used as a source of crude pentosan. For some experiments these were purified by removing the soluble starch with α-amylase as described by Kundig *et al.*[3]

* Part II: *J. Sci. Fd Agric.*, 1964, **15**, 834.

† For the purpose of this work, flour pentosans are defined as the non-dialysable polysaccharides contained in a heated water extract of flour. Such preparations contain approximately 55% α-glucosan as well as some galactose and protein.[3]