Amides of Vegetable Origin. Part IV. The Nature of Pellitorine and Anacyclin.*

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Pellitorine, a crystalline insecticidal substance isolated from *Anacyclus pyrethrum* DC. roots and hitherto accepted as a pure compound, is shown to contain at least three substances. Hydrogenated pellitorine, when hydrolysed, gives a mixture resolvable by reversed-phase chromatography into decanoic, dodecanoic, and tetradecanoic acid. *N-iso*Butyldeca-*trans*-2 : *trans*-4-dienamide is a major component of pellitorine: the other components must have closely related structures. Since pellitorine constitutes only a small part of the insecticidal amide fraction of the roots, and since the activity is not concentrated in it, interest shifts back to the main mixture. A preliminary examination of this is reported.

A new crystalline polyunsaturated *iso*butylamide isolated from pellitory root and named anacyclin is not a sialogogue and has low insecticidal activity. On catalytic hydrogenation it absorbs six mols. of hydrogen, yielding *N-iso*butyltetradecanamide. By spectroscopical studies and oxidative degradation, anacyclin is shown to be *N-iso*butyltetradeca-*trans*-2 : *trans*-4-diene-8 : 10-diynamide (III).

On controlled catalytic hydrogenation, anacyclin is converted into a highly effective sialogogue and insecticide, *N-iso*butyltetradeca-*trans*-2 : *trans*-4 : *cis*-8 : *cis*-10-tetraenamide.

PELLITORINE is a crystalline material (m. p. 72°) isolated, by a process involving solvent extraction and distillation, from the roots of a member of the Compositae, *Anacyclus Pyrethrum* DC. (Gulland and Hopton, *J.*, 1930, 6; for references to earlier investigations see Part II, Crombie, *J.*, 1952, 4338). Gulland and Hopton found that acidic hydrolysis yielded *iso*butylamine and that hydrogenation gave *N-iso*butyldecanamide, two mols. of hydrogen being absorbed. They therefore considered it to be an *N-iso*butyldecadienamide. Jacobson (*J. Amer. Chem. Soc.*, 1949, **71**, 366) claimed to have located the position of the two double bonds by permanganate degradation and proposed structure (I). In Part II

(I) $Pr^{n}\cdot CH{=}CH\cdot CH_2\cdot CH_2\cdot CH{=}CH\cdot CO\cdot NHBu^{i}$ $Me\cdot[CH_2]_4\cdot CH{=}CH\cdot CH{=}CH\cdot CO\cdot NHBu^{i}$ (II)

it was shown by synthesis that none of the four possible stereoisomers of structure (I) was pellitorine. The nature of this substance is now re-examined.

Three samples of pellitory root were investigated and crystalline pellitorine (m. p. 72—73°) was obtained from all. The yield of crude distilled material was 0·13—0·3% calculated on dry root but only about 5% of this could be obtained as crystalline material, m. p. 72°. Gulland and Hopton (*loc. cit.*) record a yield of 0·04% but do not make it clear if crude or crystalline material is referred to. Jacobson (*loc. cit.*) gave a crude yield of 0·14% but stated that 99% of this was then pellitorine of m. p. 72°; he has kindly supplied the author with a sample of his crude material. It now has m. p. 62° and gave pellitorine only after repeated crystallisations which resulted in much loss. In the present work, all experiments on pellitorine were carried out with material of m. p. 72—73°.

The ultra-violet light absorption data of pellitorine (Table 1) indicate that a sorbic-type chromophore is present; in consonance with this, a maleic anhydride adduct (m. p. 192°) is formed readily. Microhydrogenation indicates two double bonds and this, when taken with the identification of the product as *N-iso*butyldecanamide by Gulland and Hopton (*loc. cit.*; cf. Jacobson, *J. Amer. Chem. Soc.*, 1950, **72**, 1489) leads to (II) as the structure of pellitorine. Further, the reaction with maleic anhydride indicates a *trans-trans*-configuration. However, synthetic *N-iso*butyldeca-*trans*-2 : *trans*-4-dienamide (Crombie, *Chem. and Ind.*, 1952, 1034; following paper) had m. p. 90°, though it did not

* Part III, preceding paper.

depress that of pellitorine. Its maleic anhydride adduct (m. p. 192°) also caused no marked depression with that from pellitorine. The infra-red spectra of the two dienamides were identical, except that pellitorine contained an additional weak band at 909 cm.$^{-1}$ (34 bands were common to the two spectra and had similar intensities). Both substances possessed moderately high toxicity to *Musca domestica* L. and both were sialogogues. Pellitorine is rather more than half, and *N-iso*butyldeca-*trans*-2 : *trans*-4-dienamide about a third, as toxic as the pyrethrins tested at the same concentration. Nevertheless, pellitorine was lethal to the grain insect *Tenebrio molitor* L. in concentrations in which the synthetic *trans-trans*-amide (II) was innocuous. Two explanations for the discrepancies are possible. Either pellitorine is one of the other three stereoisomers of (II) or it is not homogeneous.

The first possibility can be disposed of. In Part V (following paper), synthesis of all stereoisomers of (II) is described and all differ widely from pellitorine in physical, spectroscopical, and physiological properties. The second possibility must be examined.

Repetition of Gulland's hydrogenation experiment revealed that the products were incompletely solid. After purification, hydropellitorine specimens had m. p.s between 32° and 36°, either raised or slightly depressed by pure *N-iso*butyldecanamide (m. p. 37—38°). Furthermore, pellitorine itself tended to give slightly higher analytical figures for carbon and lower figures for nitrogen than are required for the amide (II). Hydropellitorine was hydrolysed with ethanolic hydrochloric acid in a sealed tube and the presence of an *iso*butylamine cleavage fragment confirmed. The acidic product was chromatographed, by Howard and Martin's reversed-phase technique (*Biochem. J.*, 1950, **46**, 532), and three fatty acids were detected and estimated (Table 1). Their chromatographic characteristics indicate their identities as decanoic, dodecanoic, and tetradecanoic acid, and in the case of a related extract (see below) this has been confirmed by isolation.

TABLE 1. *Components of pellitory root.*

	Ultra-violet light absorption						Component acids, chain lengths (%)				
	M. p.	H_2 no.[1]	$\lambda_{max.}$ (mμ)	$\lambda_{inf.}$[2] mμ	$E^{1\%}_{1\,cm.}$ (max.)	$E^{1\%}_{1\,cm.}$ (infl.)[2]	C_8	C_{10}	C_{12}	C_{14}	C_{16}
Me·[CH_2]$_4$·[CH=CH]$_2$·CO·NHBu^t[4]	90°	112	258	264	1330	1200	—	100	—	—	—
Fraction A	70	114[3]	258	265	1300	1200	0·5	58·5	26	14	1
Fraction B	46—48	105[3]	258	265	1120	1020	1	49	25	25·5	0·5
Pellitorine	72	107	258	264	1350	1300	—	67	23	10	—
Anacyclin	121	48	259	265	1240	1100	—	—	—	100	—

[1] Hydrogen number, defined as the weight of substance in g. which absorbs one mole of hydrogen (see Ogg and Cooper, *Analyt. Chem.*, 1949, **21**, 1400). Determinations were made on samples of 5—10 mg. [2] When a Unicam photoelectric instrument is used, only the maximum at 258 mμ is observed. [3] Mean figure. [4] Part V (following paper). (t = *trans*).

Pellitorine is thus a sharply melting mixture of at least three amides. The light-absorption extinction coefficients indicate that all three components probably contain a fully conjugated *trans-trans*-diene *iso*butylamide chromophore, and the infra-red data (Table 2) are in agreement. A major component is undoubtedly the amide (II), but micro-hydrogenation figures allow a minor component to have more than two double bonds. The latter may be the cause of the marked toxicity to *Tenebrio molitor*, because *N-iso*butyldodeca-*trans*-2 : *trans*-4-dienamide (Crombie and Shah, unpublished work) is inactive (3% in acetone), indicating that the effect is not merely due to increasing chain length of the alkyl substituent. The potency of pellitory extract before distillation is comparable with that of pellitorine towards *Tenebrio* and as the preparation of the latter material involves great losses, interest in it, now that it is known to be heterogeneous, has receded. A preliminary examination of whole pellitory extract has therefore been made.

The root extract, after purification by solvent partition, was chromatographed on alumina. Components readily eluted by light petroleum (b. p. 40—60°)–ether were not resolved and form a related group of waxy highly insecticidal sialogogues (0·11—0·22% yield). They can be subdivided by crystallisation from light petroleum, in which they are extremely soluble, into fraction A (less soluble, m. p. 70°) and fraction B (m. p. 46—48°).

The composition of the mixture of saturated stem acids of the A fraction resembles that of pellitorine more than the B fraction which is richer in tetradecanoic acid. The melting point of the fraction A is undepressed on admixture with pellitorine, and there seems little doubt that the latter is similar material obtained by a different method of isolation.

TABLE 2. *Infra-red bands (cm.$^{-1}$)* [3] *relating to the stereochemistry of pellitorine.*

	ν-NH		ν-C=O	ν-C=C$_I$	ν-C=C$_{II}$	δ'-[CH=CH]$_2$
Me·[CH$_2$]$_4$·[CH=CH]$_2$·CO·NHBu^i	3295	3075	1625	1654	1614	994 [2]
Pellitorine	3300	3070	1625	1655 [1]	1615 [1]	995 [1,2]

[1] These bands are in the correct positions for a *trans-trans*-stereoisomer (see Part V). [2] No absorption near 960 cm.$^{-1}$ (nearest band 942 cm.$^{-1}$). A band there is characteristic of a *cis*-containing configuration (see Part V). [3] Paraffin mulls. (t = *trans*.)

Both fractions A and B readily yield diene adducts and give *iso*butylamine on hydrolysis; their ultra-violet and infra-red absorption data indicate that a fully conjugated *trans-trans*-diene *iso*butylamide chromophore enters into their structures. The B fraction is particularly effective insecticidally and as a sialogogue: it is more unstable than the A fraction or than pellitorine, though these may decompose to a gum during some weeks at 0°. Microhydrogenation, considered in relation to the stem acid composition, shows that amides containing more unsaturation than two double bonds must be present in the B fraction and, as remarked earlier, these may be highly effective insecticides.

Continued elution with ether gives, after purification, a new component, anacyclin, m. p. 121°. This is also encountered if the crude light petroleum extracts of pellitory root are set aside at 0°; because of its marked insolubility in this solvent, it may then crystallise. This insolubility makes it simple to ascertain that the above A and B fractions are quite free from anacyclin. Despite its unusual properties, the compound is an aliphatic *iso*butylamide and its constitution and stereochemistry are elucidated below. Anacyclin is very soluble in chloroform or carbon tetrachloride but, in contrast to pellitorine or the A and B fractions, is only slightly toxic towards *T. molitor* under the usual conditions of test. It is optically inactive.

Analysis and molecular-weight determination of anacyclin lead to the empirical formula $C_{18}H_{25}ON$. The infra-red spectrum shows it to be a monosubtituted amide (NH stretching, 3300, 3060; amide A carbonyl 1625; amide B 1544 cm.$^{-1}$), which contains αβ-conjugated diene unsaturation (1655 and 1612 cm.$^{-1}$ strong). On microhydrogenation in acetic acid six mols. of hydrogen were absorbed in the presence of a platinum catalyst. Similar results were obtained on a macro-scale with palladium–charcoal in ethyl acetate and the product, dodecahydroanacyclin (m. p. 66°) was isolated in excellent yield. This is a saturated monosubstituted amide (infra-red absorption). On hydrolysis with ethanolic hydrochloric acid it gave *iso*butylamine hydrochloride and tetradecanoic acid. Dodecahydroanacyclin must therefore be the previously undescribed *N-iso*butyltetradecanamide: this was confirmed by synthesis.

Hydrolysis of anacyclin itself also gave *iso*butylamine hydrochloride so the unsaturation must all be located in the chain of the acid. Ultra-violet light absorption indicates that two double bonds are conjugated with the amide grouping (Table 3). This leaves unsaturation equivalent to four mols. of hydrogen to be placed in the rest of the chain. No vinyl grouping is present (infra-red spectrum) and the ultra-violet absorption makes it possible to rule out any system of conjugated double bonds other than that mentioned.

Anacyclin readily forms a maleic anhydride adduct which, as expected, absorbs five mols. of hydrogen when catalytically hydrogenated. The adduct has been of great value in determining the nature and position of the remaining unsaturation. In this adduct the 2:4-dienamide-type of chromophore is totally eliminated and determination of the ultra-violet absorption spectrum reveals the low-intensity absorption characteristic of a conjugated diyne which is not quite pure: some 4% of enediyne is indicated (calculated from the ε data for deca-2-ene-4:6-diynol at 282·5 mμ, given in Table 3). Repeated crystallisations have so far failed to eliminate this. Examination of the spectrum of the maleic anhydride adduct of *N-iso*butyldeca-*trans*-2:*trans*-4-dienamide showed that no

interference with the detection of the diyne system is caused by maleic adduct formation. Presence of the diyne system is confirmed by the infra-red spectrum of both anacyclin and its maleic anhydride adduct. In thick films a doublet is found in the C≡C stretching region (Table 4). These vibrations are weak and barely detectable in paraffin mulls of the concentration and thickness generally employed in work on amides. Allenic vibrations could not be detected.

TABLE 3. *Ultra-violet light absorption (mμ) of anacyclin and related compounds.*

Anacyclin [1]	λ 230, ε 10,000; $\lambda_{max.}$ 259, ε 33,500					
Tetrahydroanacyclin [1]	λ 230, ε 24,000; $\lambda_{max.}$ 259, ε 30,500					
$Me[CH_2]_4 \cdot CH^t{=}CH \cdot CH^t{=}CH \cdot CO \cdot NHBu^i$ [1]	$\lambda_{max.}$ 259, ε 29,500					
Anacyclin maleic anhydride adduct	$\lambda_{max.}$	227	237	252	266	281
	ε	1850	1300	1100	1000	850
$Me \cdot [CH_2]_3 \cdot C{\equiv}C \cdot C{\equiv}C \cdot [CH_2]_3 \cdot Me$ [2]	$\lambda_{max.}$	228	239·5	254	—	—
	ε	440	390	240		
$CH_2{=}CH \cdot [CH_2]_4 \cdot C{\equiv}C \cdot C{\equiv}C \cdot [CH_2]_7 \cdot CO \cdot NHR'$ [3, 4]	$\lambda_{max.}$	227	238	251	—	—
	ε	640	445	180		
$Me \cdot [CH_2]_2 \cdot C{\equiv}C \cdot C{\equiv}C \cdot CH{=}CH \cdot CH_2 \cdot OH$ [5]	$\lambda_{max.}$	—	239·5	252	266·5	282·5
	ε	—	7300	14,600	21,850	19,440

[1] An inflexion at ~265 mμ is detectable. [2] Armitage, Cook, Entwistle, Jones, and Whiting, *J.*, 1952, 1998. [3] Black and Weedon, *J.*, 1953, 1785. [4] R = $CH_2 \cdot CH_2 \cdot OH$. [5] Bruun, Haug, and Sörensen, *Acta Chem. Scand.*, 1950, **4**, 850.

TABLE 4. *Acetylene stretching frequencies (cm.$^{-1}$).*

Anacyclin [1]	2233 w	2206 vw
,, maleic adduct [1]	2243 w	2208 vw
$Me \cdot [CH_2]_5 \cdot C{\equiv}C \cdot C{\equiv}C \cdot [CH_2]_8 \cdot CO_2R$ [1, 2]	2253 w	2228 w, 2203 vw
$C_5H_{11} \cdot C{\equiv}C \cdot C{\equiv}C \cdot CO_2Me$ [3]	2251 s	2224 m
$C_5H_{11} \cdot C{\equiv}C \cdot CH^t{=}CH \cdot CO_2Et$ [3] (t = *trans*.)	—	2212 s

[1] Paraffin mulls. [2] R = *p*-$C_6H_4Br \cdot CO \cdot CH_2$. The author is grateful to Dr. B. C. L. Weedon for this sample. [3] Part V, liquid film.

The conjugated diyne system must be situated between $C_{(7)}$ and $C_{(13)}$ and its position was found by oxidative degradation. Ozonolysis caused resinification but permanganate degradation proved to be satisfactory. *N-iso*Butyloxamic acid, succinic acid, and a derivative of butyric acid were isolated. The presence of a diyne system makes intelligible the insolubility in light petroleum and the tendency to become pink in light (cf. Armitage, Cook, Entwistle, Jones, and Whiting, *J.*, 1952, 1998). On alkali isomerisation, the 259-mμ maximum of anacyclin disappeared and was replaced by a new maximum at 302 mμ. This wave-length, however, is rather short for a conjugated yne-trieneamide chromophore such as might be expected to develop under these conditions.

Three pieces of evidence show that the 2 : 4-diene system has the *trans-trans*-configuration. First, the ease of formation of a maleic adduct is characteristic, and, secondly, the infra-red trace shows only one peak at 996 cm.$^{-1}$ without a second in the 960—965 cm.$^{-1}$

$$Me \cdot [CH_2]_2 \cdot C{\equiv}C \cdot C{\equiv}C \cdot CH_2 \cdot CH_2 \cdot CH^t{=}CH \cdot CH^t{=}CH \cdot CO \cdot NHBu^i \quad \text{(III)}$$

region (the nearest is at 943 cm.$^{-1}$). Finally the two C=C stretching bands (vibrational interaction) fall in the expected positions (values given above; see Part V for a full examination of these questions). Anacyclin is, therefore, *N-iso*butyltetradeca-*trans*-2 : *trans*-4-diene-8 : 10-diynamide (III).

In recent years numerous acetylenic compounds have been found in a number of flowering plants and fungi: in particular, straight chain conjugated enyne esters have been obtained from the Compositae family to which *Anacyclus pyrethrum* belongs (Williams, Smirnow, and Goljmow, *J. Gen. Chem., U.S.S.R.*, 1935, **5**, 1195; Sörensen and Stene, *Annalen*, 1941, **549**, 80; Holman and Sörensen, *Acta Chem. Scand.*, 1950, **4**, 416; Stavolt and Sörensen, *ibid.*, pp. 1567, 1575; Baalsrud, Holme, Nestvold, Pliva, Sörensen, and Sörensen, *ibid.*, 1952, **6**, 883; Christiansen and Sörensen, *ibid.*, p. 893). The C_{18} fatty acid

erythrogenic acid obtained from the fruit kernel oil of *Ongokea Gore* Engler contains, like anacyclin, an isolated diyne linkage, though it belongs to another family (Castille, *Annalen*, 1940, **543**, 104; Steger and van Loon, *Rec. Trav. chim.*, 1940, **59**, 1156; Armitage *et al.*, *loc. cit.*; Black and Weedon, *J.*, 1953, 1785). It too tends to be contaminated with small amounts of material having a conjugated enediyne chromophore. An interesting feature of the structure of anacyclin is the dimethylene bridge between two unsaturated systems. This is characteristic of various insecticidal *iso*butylamides such as *neo*herculin (preceding paper), affinin (Acree, Jacobson, and Haller, *J. Org. Chem.*, 1945, **10**, 236, 449), scabrin (Jacobson, *J. Amer. Chem. Soc.*, 1951, **73**, 100), and sanshoöl I and II (Aihiara, *J. Pharm. Soc. Japan*, 1950, **70**, 43, 47). The system ·CH=CH·CH_2·CH_2·CH=CH· is also found in certain fish oils.

Existing evidence (Part III and above) suggests that unsaturation conjugated with the *iso*butylamide linkage is all-*trans* in at least some of the natural insecticidally active compounds of this class. On the other hand, *cis*-containing arrangements are desirable for "internal" unsaturation not conjugated with the amide grouping (Part III). Further, synthetical studies (Part V) show that, for the system

$$CH_3\cdot[CH_2]_4\cdot CH{=}CH\cdot CH{=}CH\cdot CO\cdot NHBu^i,$$

the *trans*-2 : *trans*-4- is ten times more active than the *trans*-2 : *cis*-4-compound towards *Musca domestica*. Anacyclin was therefore partially hydrogenated with lead–quinoline poisoned catalyst (Lindlar, *Helv. Chim. Acta*, 1952, **35**, 446) until two mols. of hydrogen had been absorbed. The catalyst is largely selective and stereospecific for the hydrogenation of a diyne to a *cis-cis*-diene system (see Part V) so the product is formulated as *N-iso*butyltetradeca-*trans*-2 : *trans*-4 : *cis*-8 : *cis*-10-tetraenamide. It was not further purified, but the light absorption agrees with this structure, ε at 230 mμ having increased by 14,000 (Table 3) because the weakly absorbing diyne has been replaced by a *cis-cis*-diene chromophore. This new compound was very soluble in light petroleum and had strong sialogogue effect and insecticidal activity towards *Tenebrio molitor*. Like herculin and the fractions A and B (p. 1005) it rapidly deteriorated to a brown gum. The complete structure *and* the stereochemistry of an *iso*butylamide with high activity towards *Tenebrio* are thus known for the first time. It is conceivable that such a compound, or a stereoisomer about the 8 : 10-double bonds, might account for the *Tenebrio* activity of the fractions A and B.

Experimental

Microanalyses were carried out in the microanalytical laboratories (Mr. F. H. Oliver) and ultra-violet light absorption data measured in the spectrographic laboratories (Mrs. I. Boston) of Imperial College. The latter data were obtained with a Hilger medium quartz instrument and absolute ethanol as solvent.

Examination of Pellitory Root.—Ground root (8 kg.) was continuously extracted with light petroleum (b. p. 40—60°) in a large Soxhlet-type apparatus. When the extract was set aside for 2 weeks at 0° (vol., 1·5 l.), a yellow oil separated, together with crystals (1·1 g.; m. p. 109°). Five recrystallisations of the latter, from light petroleum (b. p. 40—60°) containing a little chloroform or carbon tetrachloride, gave anacyclin as white needles, m. p. 121°.

The main light petroleum solution was concentrated to 700 ml. and extracted with nitromethane (4 × 250; 5 × 100 ml.). These extracts were concentrated (500 ml.) and cooled at 0° for some days. Solid (2·6 g.), m. p. 58—62°, was deposited which from its odour, taste, and appearance was similar to the A and B mixtures described below. It was returned to the nitromethane solution, and the solvent removed *in vacuo*. The pale yellow residue was dissolved in ether and washed with 2N-hydrochloric acid, and then with water. After drying and evaporation of the ether, a cream-coloured waxy solid (15·6 g., 0·195%) remained [absorption max. at 258; inflexion at 266 mμ ($E^{1\%}_{1\,cm.}$ 910, 870)]. This is referred to below as "purified pellitory extract."

Root from a different source (3·63 kg.) was ground immediately before extraction. The extracts deposited 2·6 g. of crude anacyclin, and purified pellitory extract was obtained in 0·37% yield.

Pellitorine.—Purified pellitory extract (4·2 g.) was distilled (b. p. 158—195°/0·07 mm.). The pale yellow distillate (3·45 g.), collected as one fraction, crystallised as a waxy mass of

needles (m. p. 48—53°) (Found: C, 75·75; H, 11·05; N, 5·05%; H_2 no., 114). Light absorption: $\lambda_{max.}$ 258, $\lambda_{infl.}$ 266 mμ ($E^{1\%}_{1cm.}$ 1070, 1020). This was crystallised from light petroleum (b. p. 40—60°; *ca.* 5 ml.) and after three days at 0° the crystals were collected by filtration at 0°. Three further crops were obtained, after concentration, by similar slow crystallisations. This product (300 mg.; m. p. 63—66°) was twice crystallised from light petroleum in long silky needles and then melted sharply at 72° (184 mg.) (Found: C, 75·55; H, 11·1; N, 6·0. $C_{14}H_{25}ON$ requires C, 75·25; H, 11·3; N, 6·25%; H_2 no., 112. $C_{16}H_{29}ON$ requires C, 76·5; H, 11·65; N, 5·55%; H_2 no., 126). For other data see Table 1. A specimen obtained from another batch of root had m. p. 73° (Found: C, 75·8, 75·9; H, 11·15, 11·20; N, 5·65, 6·1%. H_2 no., 111) and light absorption $\lambda_{max.}$ 256, $\lambda_{infl.}$ 264 mμ ($E^{1\%}_{1cm.}$ 1450; 1350).

A specimen received from Dr. Jacobson had m. p. 62—63°, raised by three crystallisations (with much loss) to 73° [light absorption: $\lambda_{max.}$ 257, $\lambda_{infl.}$ 265 mμ ($E^{1\%}_{1cm.}$ 1350, 1300)], undepressed on admixture with the pellitorine specimens described above.

Pellitorine is a potent sialogogue. A 3·1% solution in acetone, when tested by topical application of measured drops under defined conditions, against adult *Tenebrio molitor* L., caused 45% immobilisation after 24 hr. (see Report of Rothamsted Exp. Station, 1952, p. 103). It formed a maleic anhydride *adduct* (Found: C, 67·9; H, 8·9. $C_{18}H_{27}O_4N$ requires C, 67·3; H, 8·5. $C_{20}H_{31}O_4N$ requires C, 68·7; H, 8·95%), which crystallised from benzene in needles, m. p. 192° (unsharp), not depressed on admixture with the maleic anhydride adduct of *N-iso*butyldeca-*trans*-2 : *trans*-4-dienamide.

Hydrogenation of Pellitorine.—The material was completely hydrogenated in ethyl acetate in presence of palladium–calcium carbonate. After filtration and evaporation *in vacuo*, the product was semisolid at 25°; after being pressed on a porous tile it had m. p. 34—36°, depressed to 31—32° if admixed with pure *N-iso*butyldecanamide (m. p. 37—38°). Another specimen had m. p. 32°, unaltered by admixture with *N-iso*butyldecanamide.

Hydrolysis of Hydropellitorine.—Pellitorine (92 mg.) was hydrogenated as described above and the hydropellitorine heated at 100° for 2 days with ethanol (2 ml.) and concentrated hydrochloric acid (0·7 ml.) in a sealed tube. The mixture was then diluted with water and extracted with ether. Evaporation of the aqueous phase and recrystallisations from ethyl acetate gave *iso*butylamine hydrochloride, m. p. 171—172° undepressed by an authentic specimen of m. p. 174°. The ethereal extract was evaporated and hydrolysed by heating it under reflux with 2N-sodium hydroxide. Neutral material was removed by ether-extraction, and the acids, liberated by acidification, were isolated in the usual way. After removal of traces of ether at 0·02 mm., the acidic hydrolysis product was chromatographed on a column (30 cm. × 12 mm.) of water-repellent kieselguhr impregnated with liquid paraffin (B.P.) and eluted with solutions of acetone in water (successively 35%, 45%, 50%, 60%, 70% of acetone). Three components were separated (in the 45%, 50%, and 60% solutions): these correspond to decanoic, dodecanoic, and tetradecanoic acid, as shown by chromatography of a standard mixture (see also the isolation below). The progress of the chromatogram and composition of the mixture was followed by microtitration of three hundred successive 2-ml. portions of eluant to bromothymol-blue (see Table 1).

Chromatography of Purified Pellitory Extract.—The extract (11·2 g.) was dissolved in 5 : 1 light petroleum (b. p. 40—60°) and ether, and the brown solution chromatographed on alumina (30 × 3 cm.). After elimination of a trace of a yellowish substance, the material eluted by the above solvent (650 ml.), and then by light petroleum (b. p. 40—60°) and ether (1 : 1; 500 ml.), was collected in five fractions (total 6·14 gm.). These are referred to as X. Further elution with pure ether (750 ml.) gave crude orange material (3·95 gm.) differing from the above in its low sialogogue effect, low insecticidal effectiveness (*Tenebrio*), and low solubility in light petroleum. The latter crude fractions were repeatedly extracted with light petroleum (b. p. 60—80°), and the extracted solid (1·4 g.) was crystallised from this solvent containing a little chloroform to give anacyclin, m. p. 121° (0·3 g.). Resinous material remained. Further elution with ether–ethanol (4 : 1) gave a clear brown resin with a characteristic odour which gave no crystals when extracted with light petroleum (b. p. 60—80°).

Examination of the Material X.—The fractions X were white or pale yellow waxy solids of m. p. 38—55°, extremely soluble in light petroleum (b. p. 40—60°), and highly potent insecticides and sialogogues. A specimen of the united fractions (m. p. 49°) caused immobilisation of 96% of the insects after 24 hr., when applied to *Tenebrio* at a concentration of 3·15% (in acetone). They were very unstable and changed to dark resins in a few days at 0° in stoppered tubes under nitrogen. They are best kept in solution in light petroleum or ether. On their dissolution in a small quantity of light petroleum (10 ml./g.; b. p. 40—60°) and storage for 3 days at

0°, sheaves of needles were deposited. The material X (6·14 g.) gave 0·61 g. (m. p. 70°). This material is termed A (0·01% calc. on dried root), and the petroleum-soluble fraction B (0·096%).

Fraction A.—Admixture with pellitorine (m. p. 72—73°) did not depress the m. p. (Found: C, 75·0, 75·75; H, 11·5, 11·2; N, 6·05, 6·0%. H_2 no., 112, 116). Light absorption: $\lambda_{max.}$ 258, $\lambda_{infl.}$ 265 mμ ($E^{1\%}_{1\,cm.}$ 1400, 1300) (see also Table 1). The material A (139 mg.) was hydrogenated and hydrolysed with alcoholic hydrochloric acid as described under pellitorine. The hydrochloride of the basic fraction (57 mg.), after crystallisations from ethyl acetate, had m. p. 171°, undepressed on admixture with *iso*butylamine hydrochloride, m. p. 173°. The acids (used, 40·3 mg.) were subjected to reversed-phase chromatography and the approximate composition is given in the Table.

Fraction B.—This was a pale yellow waxy solid, m. p. 46—48° (Found: C, 75·65, 76·1; H, 11·25, 11·2; N, 5·5, 5·65%; H_2 no., 108, 102). Light absorption: $\lambda_{max.}$ 258, $\lambda_{infl.}$ 265 mμ ($E^{1\%}_{1\,cm.}$ 1100, 1000). See also Table 1. The material B (570 mg.) was hydrogenated, hydrolysed with ethanolic hydrochloric acid, and worked up as above. The crude amine hydrochloride (125 mg.) was thrice crystallised from ethyl acetate, forming plates, m. p. 170°, undepressed by authentic *iso*butylamine hydrochloride. The solid acids, when analysed by the chromatographic procedure, had the composition given in Table 1.

In an earlier experiment, a fraction X (m. p. 48—50°) [Found: C, 76·35; H, 11·3; N, 5·45%; H_2 no., 118. Light absorption: $\lambda_{max.}$ 258, $\lambda_{infl.}$ 264 mμ ($E^{1\%}_{1\,cm.}$ 1070, 920)] was hydrogenated (the product had m. p. 44—49°) and hydrolysed, and the acids (Found: C, 70·65; H, 11·95%; equiv., 190) were chromatographed. The fundamental acid composition was decanoic 40%, dodecanoic 33%, and tetradecanoic 27%. Solutions of the first two bands from the chromatogram were separately evaporated, and acidified, and the acid was collected with ether. Indicator (bromothymol-blue), which contaminated the acid, was removed by distilled water and the acid converted into its *p*-bromophenacyl ester. The derivative from the first acid was *p*-bromophenacyl decanoate, m. p. and mixed m. p. 65·5° (shining plates from ethanol). The second fraction gave *p*-bromophenacyl dodecanoate, m. p. and mixed m. p. 72°, markedly depressed by the derivatives of decanoic and tetradecanoic acid. The presence of tetradecanoic acid was established by distillation of the crude mixture of saturated acids (260 mg.) isolated by hydrogenation and hydrolysis of fraction X (540 mg.). After elimination of material, b. p. 118—120°/0·2 mm., n_D^{25} 1·4372, the temperature rose to 132° and a fraction was collected which solidified in the receiver (63 mg.) and, crystallised three times from ethanol–water, had m. p. 55—56°, undepressed by tetradecanoic acid.

Isolation of Anacyclin.—*Anacyclin* is deposited from light petroleum (b. p. 40—60°) extracts of pellitory root when set aside at 0°. The remainder of the material may be isolated from the extract, after partition with nitromethane, by chromatography (see above). Total yields are 0·03—0·07% based on dry root. Anacyclin is sparingly soluble in light petroleum: when repeatedly crystallised from this (b. p. 60—80°) it had m. p. 116° but the m. p. could be raised to 121° by further crystallisations from light petroleum containing a little chloroform, carbon tetrachloride, or ethyl acetate [Found: C, 79·7, 79·85, 79·5; H, 9·6, 9·5, 9·2; N, 5·4; 5·5, 5·2%; *M* (Rast), 284. $C_{18}H_{25}ON$ requires C, 79·7; H, 9·3; N, 5·15%; *M*, 271·4. Microhydrogenation no., 47·6, 47·7, 46·7, equiv. to 5·7, 5·7, 5·8 H_2]. For ultra-violet light absorption see Table 2. The substance crystallises in white needles but in stoppered tubes at 0° it becomes yellow in a few days, and the m. p. falls. The crystals are conveniently stored under a layer of light petroleum at 0°. When dried at 25° or 70° in light, a specimen became salmon pink (even at 0·5 mm.). A 3% solution in acetone, when tested against *Tenebrio molitor* by the measured-drop technique, caused only 10% mortality. This may be a reflexion of its low lipoid solubility.

Dodecahydroanacyclin.—Anacyclin (332 mg.) was completely hydrogenated in methyl acetate solution in presence of 5% palladium and charcoal (200 mg.). (Absorption of hydrogen 166 ml. at N.T.P.; calc. for $6H_2$, 165 ml.) Kieselguhr was added, the catalyst removed by filtration and the solution concentrated. Colourless needles (267 mg.), m. p. 66°, of the *decahydro-compound* were deposited [Found: C, 76·5; H, 13·5; N, 4·9%; *M* (Rast) 269. $C_{18}H_{37}ON$ requires C, 76·25; H, 13·15; N, 4·95%; *M*, 283·5]. The infra-red spectrum (paraffin mull) showed bands at 3310, 3075 (NH stretching), 1641 (amide A C=O) and 1548 (amide B) cm.$^{-1}$.

Hydrolysis of Dodecahydroanacyclin.—The dodecahydro-compound (210 mg.) was heated at 80—105° in a sealed tube with ethanol (8 ml.) and concentrated hydrochloric acid (2 ml.) for 3 days, then diluted with water and extracted with ether. Evaporation of the aqueous phase gave *iso*butylamine hydrochloride (88 mg.) which when crystallised from ethyl acetate had m. p. 173—174°, undepressed by an authentic specimen m. p. 174—175° (Found: Cl, 32·25. Calc. for $C_4H_{12}NCl$: Cl, 32·25%). The ethereal extract was evaporated and acidic material extracted

with 2N-sodium hydroxide. Liberation with 2N-sulphuric acid gave crude tetradecanoic acid (73 mg.) which was converted into its *p*-bromophenacyl ester, plates (from ethanol), m. p. and mixed m. p. 80°.

N-iso*Butyltetradecanamide*.—This was prepared in the usual way from tetradecanoyl chloride and *iso*butylamine and crystallised from ethyl acetate in needles, m. p. 66°. When admixed with dodecahydroanacyclin, there was no depression of m. p.

Maleic Anhydride Adduct of Anacyclin.—Anacyclin (90 mg.), maleic anhydride (10 mg.), and benzene (0·6 ml.) were heated together for 18 hr. in a sealed tube at 80—100°. The material (77 mg.) which crystallised from the brown solution on cooling was washed with cold benzene and then crystallised from this solvent, after treatment with charcoal. The *adduct* was obtained in needles, m. p. 193° (decomp.) (Found: C, 71·7, 70·5; H, 7·6, 7·35. $C_{22}H_{27}O_4N$ requires C, 71·5; H, 7·35%. Microhydrogenation no., 82·1, equiv. to 4·5H_2). For light absorption, see Table 2.

Tetrahydroanacyclin.—Anacyclin (99·8 mg.) was stirred, in ethyl acetate solution containing a drop of quinoline, with lead-poisoned palladium catalyst (200 mg.; Lindlar, *loc. cit.*) under hydrogen, until 15·88 ml. (N.T.P.) were absorbed (Calc. for 2H_2: 16·5 ml.). After filtration, solvent was evaporated *in vacuo* and the residue dissolved in ether and washed with dilute sulphuric acid and then water. The solution was dried and evaporated; the unpurified residue, heated at 100°/1 mm., crystallised on cooling as rosettes of waxy needles, m. p. 40°. This material was used for the light absorption determination. It was a strong sialogogue and, when tested against *Tenebrio* as a 3% solution in acetone, caused 100% immobilisation.

Oxidation of Anacyclin.—Potassium permanganate (1·4 g.) was added a little at a time to anacyclin (260 mg.), suspended in water (25 ml.) and acetone (5 ml.) at 50°. All was decolourised and the precipitated manganese dioxide was removed and well washed with hot water. The solution was acidified and steam-distilled, 220 ml. being collected. The distillate required 14·0 ml. of 0·1N-alkali for neutralisation. It was then again acidified and steam-distilled, 20-ml. portions being collected and titrated. The first five, containing the most volatile material, required 7·6 ml. of 0·1N-alkali (the theor. yield of butyric acid would require 9·6 ml.). After concentration, this gave an *S*-benzylthiuronium salt, m. p. 145°. Donleavy (*J. Amer. Chem. Soc.*, 1936, **58**, 1004) gives m. p. 146° for the derivative of *n*-butyric acid. However, an authentic specimen was found to give values as high as 153° depending on the rate of heating. Berger (*Acta Chem. Scand.*, 1954, **8**, 427) has recently commented on the similar behaviour of a range of *S*-benzylthiuronium salts and gives m. p. values from 144° to 150° for the butyric acid derivative. The m. p. of the derivative of the oxidation product was undepressed on admixture with that of *n*-butyric acid, but when admixed with *S*-benzylthiuronium propionate (m. p. 154°) gave a substantial depression.

The solution of non-steam volatile oxidation products (75 ml.) was continuously extracted with ether for 4 days. The ether was evaporated, leaving a brown mass of crystals. This was extracted with boiling light petroleum (4 × 30 ml.; b. p. 40—60°). Evaporation of the extract and recrystallisation from the same solvent gave *N-iso*butyloxamic acid, m. p. 107°, undepressed by an authentic specimen (m. p. 108°; cf. Malbot, *Compt. rend.*, 1887, **104**, 229) prepared by oxidation of *N-iso*butylsorbamide by the above procedure.

The residue, after extraction with light petroleum, was fractionally sublimed at 0·02 mm. and the residue remaining in the apparatus (crystals and resin) was thrice crystallised from ethyl acetate in needles, m. p. 185°, undepressed on admixture with authentic succinic acid (m. p. 186°) but heavily depressed by oxalic acid [m. p. 181° (decomp.)].

Alkaline Treatment of Anacyclin.—The amide (20 mg.) was refluxed for 15 min. with 25% aqueous potassium hydroxide. Slight darkening occurred. The cooled solution was extracted with ether, and the extracts were evaporated. Crystallisation from light petroleum (b. p. 40—60°)–ether gave unchanged anacyclin (mixed m. p.).

Anacyclin (9·8 mg.) was sealed in a glass tube with 20% potassium hydroxide in ethylene glycol (1 ml.) and heated at 180° for 20 min. A brown colour developed. The solution was diluted with ethanol, and an aliquot part used for light absorption measurements: $\lambda_{max.}$ 302 mμ ($E^{1\%}_{1cm.}$ 350). A blank determination was carried out.

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