

Amides of Vegetable Origin. Part III.* Structure and Stereochemistry of *neoHerculin*.

By L. CROMBIE.

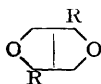
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neoHerculin, a pungent insecticidal principle from *Zanthoxylum clava-herculis* L., is obtained as a pure, but extremely unstable, compound and shown to have the molecular formula $C_{16}H_{25}ON$. It contains four double bonds, three of them in a conjugated triene system. Complete hydrogenation gives *N-isobutyldodecanamide*. The fourth double bond is shown by spectroscopical evidence to be in the 2-position, and the position of the triene system in the chain located by oxidative degradation. *neoHerculin* is *N-isobutyldodeca-2:6:8:10-tetraenamamide*. Its stereochemistry is discussed.

Another pungent and insecticidal fraction has been isolated and preliminary results suggest that this too contains *N-isobutylpolyeneamides*.

In Part I* it was shown that the bark of *Zanthoxylum clava-herculis* L. (family Rutaceae) † (southern prickly ash) contains an unstable secondary amide. This compound, named *neoherculin*, is highly insecticidal and has ultra-violet light absorption characteristic of a conjugated triene. The amide has now been obtained pure and its structure determined. A preliminary note concerning its structure has been published (Crombie, *Nature*, 1954, 174, 833).

Southern prickly ash bark was extracted with light petroleum (b. p. 40–60°) and the active material concentrated by partition with nitromethane. Purification of the nitromethane extract led to gradual elimination of two important components of the bark, asarinin (I) and *N-2-p-methoxyphenylethyl-N-methylcinnamamide* (II), subsequently



(I; R = 3:4:1- $CH_2O_2C_6H_5$)

p-MeO-C₆H₄-CH₂-CH₂-NMe-CO-CH:CHPh
(II)

referred to as herclavin (for references see Part I). These are readily separable by crystallisations from ethanol and were obtained in 0.26% and 0.38% yield respectively. No other compounds could be detected. After all the asarinin and herclavin had been removed, the mother-liquors, on cooling to -50°, yielded *neoherculin* (0.025% yield).

neoHerculin is crystalline (m. p. 70°) but unstable in air. It is highly insecticidal to *Musca domestica* L. and *Tenebrio molitor* L. (adults) and is a strong sialogogue with a

MeCH=CH·CH=CH·CH=CH·[CH ₂] ₃ ·CH=CH·CO·NHBu ¹ , ...	λ 227 mμ	λ _{max.} 259	269	280 mμ
<i>neoHerculin</i>	ε 13,000	ε 31,500	43,500	36,000
(α)-Me·[CH ₂] ₃ ·CH ^t ·CH=CH ^t ·CH=CH ^c ·CH=CH ^c ·[CH ₂] ₇ ·CO ₂ H ¹	λ 226 mμ	λ _{max.} 261	271	281 mμ
	ε 4,000	ε 36,000	47,000	38,000
(β)-Me·[CH ₂] ₃ ·CH ^t ·CH=CH ^t ·CH=CH ^c ·CH=CH ^c ·[CH ₂] ₇ ·CO ₂ H ¹	λ 226 mμ	λ _{max.} 259	268	279 mμ
	ε 5,500	ε 47,000	61,000	49,000
Me·[CH ₂] ₆ ·CH ^t ·CH·CO·NHBu ¹ *	λ _{max.} 226 mμ			
	ε 10,300			
Me·[CH ₂] ₄ ·CH ^c ·CH·CO·NHBu ¹ *	λ _{max.} 226 mμ			
	ε 10,000			

c = *cis*; t = *trans*. ¹ Crombie and Taylor, *J.*, 1954, 2816. ² Crombie, Part I.

characteristic burning taste. It has an empirical formula $C_{16}H_{25}ON$ and microhydrogenation reveals four double bonds. The infra-red spectrum (paraffin mull) shows it to be a monosubstituted amide [NH(stretching), 3260 cm^{-1} , 3070 cm^{-1} ; amide A (carbonyl), 1626 cm^{-1} ; amide B, 1559 cm^{-1}]. On hydrolysis with ethanolic hydrochloric acid, *isobutylamine* was obtained, but because of its instability the polyunsaturated acid was

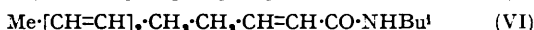
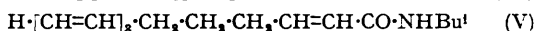
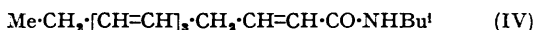
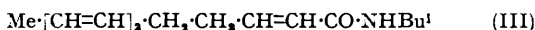
* Parts I and II, *J.*, 1952, 2997, 4338.

† The spelling *Xanthoxylum*, which is frequently encountered in the literature, was used in Part I. The name originally given to the genus was *Zanthoxylum*, and by convention this spelling is correct. Dr. D. A. H. Taylor kindly provided this information.

not isolated. Direct comparison of octahydro*neo*herculin with synthetic *N*-isobutyl-dodecanamide showed them to be identical, thus establishing an unbranched chain for the acid fragment.

Since a saturated amine is produced by hydrolysis of *neo*herculin, all four double bonds lie in the chain of the acid. Ultra-violet light absorption (Table) shows that three of these are conjugated and that this unit is not further conjugated with the amide function. Nevertheless, the intensity of the C=C stretching vibration in the infra-red demonstrates that the amide grouping has $\alpha\beta$ -unsaturation. (Experiments on the elaeostearic acids confirm that an internal triene system gives only weak absorption.) The position of the C=O stretching frequency (1626 cm^{-1}) is also in agreement. It was shown in Parts I and II that $\alpha\beta$ -unsaturation lowers the stretching frequency from 1642—1644 cm^{-1} to 1626—1634 cm^{-1} in a series of closely related isobutylamides. In confirmation, the ultra-violet absorption data (see Table) show that, when compared with an $\alpha\beta$ -saturated conjugated triene (β -elaestearic acid), *neo*herculin has a much higher ϵ value at 227 $m\mu$ (difference ~ 9000); this agrees reasonably with the ϵ at 227 $m\mu$ of simpler $\alpha\beta$ -unsaturated isobutylamides.

The results leave structures (III—V) as possible for *neo*herculin, and of them *N*-isobutyl-dodeca-2 : 6 : 8 : 10-tetraenamide (III) is correct because ozonolysis yields acetaldehyde. *neo*Herculin is thus closely related to another insecticidal sialogogue, affinin (VI), which was isolated from *Heliopsis longipes* (A. Gray) Blake by Acree, Jacobson, and Haller (*J. Org. Chem.*, 1945, 10, 235, 449; 1947, 12, 731).



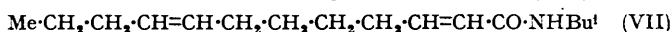
The geometrical stereochemistry of *neo*herculin can be partly, but not completely, settled. The C=C stretching vibration (1670 cm^{-1}) of the $\alpha\beta$ -double bond lies in a region characteristic of *trans*- $\alpha\beta$ -unsaturated amides (1668—1675 cm^{-1}), the corresponding *cis*-compounds having absorption at 1652—1660 cm^{-1} . Five examples of each type are given in previous Parts of this series. The strong absorption at 993 cm^{-1} in the CH=CH out-of-plane deformation region of the infra-red is due to triene and the second, moderately strong peak in this region (970 cm^{-1}) might be assigned to the *trans*-double bond $\alpha\beta$ to the carboxamide grouping (this usually appears at *ca.* 977 cm^{-1} and is absent in the *cis*-compound); however, the possibility of absorption in this region by the particular configuration of the triene present in *neo*herculin makes the assignment uncertain (α -elaestearic and α -kamfolenic acid each have absorption at 991—993 and 967 cm^{-1} . Absence of an absorption maximum of medium intensity at 818 cm^{-1} also agrees with an $\alpha\beta$ -*trans*-linkage (see Parts I and II, *loc. cit.*). A maleic anhydride adduct was not formed by *neo*herculin at 100° and this suggests that there are not two contiguous *trans*-double bonds. The relatively low extinction coefficients in the ultra-violet (Table) also denote the presence of one or more *cis*-linkages. Nothing is known of the stereochemistry of affinin, but the report that it too does not give a maleic adduct (Acree, Jacobson, and Haller, *loc. cit.*) indicates that the diene system is not *trans-trans*.*

After removal of *neo*herculin, the mother-liquors from the extract still showed high insecticidal activity. By chromatography this fraction can be resolved into three groups of crude fractions. The main one shows high activity towards *Tenebrio molitor* and is a powerful sialogogue: the other two are innocuous. No pure compound has as yet been isolated from this main group. Spectroscopic results suggest that a polyene isobutylamide, possibly containing a conjugated triene system, is responsible for the activity and the material awaits further study.

The original observation that southern prickly ash bark contains an insecticidal substance was made by La Forge, Haller, and Sullivan (*J. Amer. Chem. Soc.*, 1942, 64,

* Since this paper was submitted Jacobson (*J. Amer. Chem. Soc.*, 1954, 76, 4606) has suggested that the $\alpha\beta$ -linkage in affinin is *cis*, as a moderately strong band at 818 cm^{-1} disappears on stereomutation. The diene system is thought to be *cis-trans*, but its orientation is unknown.

187). Isolation of the active material, m. p. 59—60°, was claimed by Jacobson (*ibid.*, 1948, 70, 4234) who named it herculin and gave it structure (VII). That this could not



be so was demonstrated in Part I. From the description of the isolation and properties of herculin, it seems likely that herculin was crude *neoherculin*.

EXPERIMENTAL

Analyses and light absorption measurements (Hilger medium quartz instrument; solvent, ethanol) were carried out in the microanalytical (Mr. F. H. Oliver) and spectrographic (Mrs. I. Boston) laboratories of this Department.

Investigation of Southern Prickly Ash Bark.—Ground bark (6 kg.) was continuously extracted (cold Soxhlet-type apparatus) with light petroleum (b. p. 40—60°) for 32 hr. The extract was adjusted to 3 l. and agitated with nitromethane (300 ml.) to dissolve solids which had crystallised. The petroleum solution was then extracted with further nitromethane (2 × 300 ml.; 1 × 200 ml.; 3 × 100 ml.), and these green nitromethane extracts were united; the petroleum solution was then discarded. After removal of the nitromethane *in vacuo*, the residual oil was dissolved in ether (500 ml.) and washed with 2N-hydrochloric acid, saturated sodium hydrogen carbonate solution, and water. This dried (Na_2SO_4) extract was evaporated and repeatedly extracted with boiling light petroleum (b. p. 60—80°; 10 × 100 ml.).

The remaining dark viscous liquid was first examined. Stirring it with hot ethanol gave an extract which yielded asarinin (6.05 g. after two crystallisations and treatment with charcoal; m. p. and mixed m. p. 121—122°). The residual oil deposited large crystals when set aside for 3 weeks; these were triturated with a little ethanol and filtered off. Crystallisation from light petroleum (b. p. 60—80°)—ethyl acetate and treatment with charcoal gave herclavin, m. p. 76°, in massive well-formed crystals (5.0 g.).

The light petroleum (b. p. 60—80°) extracts (1 l.) deposited an oil at 20° which yielded asarinin (2.3 g.) and herclavin (2.5 g.). When evaporated to 400 ml. and cooled to 0° (4 hr.) the solution deposited more oil which contained asarinin (3.15 g.) and herclavin (15 g.), together with a little impure *isobutylamide* (0.15 g.; characteristic pungency). After a further reduction of the volume to 200 ml. by distillation, almost pure asarinin (0.92 g.) crystallised. On filtration and storage at 0° (18 hr.) glistening plates of pure asarinin (2.96 g.) were deposited.

As nothing more crystallised after 6 hr. at 0°, cooling was continued with solid carbon dioxide-ethanol: after some delay, a gel separated which was filtered off with difficulty and roughly dried by being pressed on a porous plate (0.20 g.; m. p. 67—69°). Further cooling gave more gel which when treated as above gave rather sticky green flakes (1.30 g.; m. p. 61—63°). These two crops were crude *neoherculin*.

Prolonged cooling at -40° yielded no more solid and after 3 days at 0° only traces of asarinin (45 mg.) and herclavin (21 mg.) were produced. Repetition of the cooling to -40° gave a green impure waxy solid (340 mg.) with characteristic pungent taste; ultra-violet absorption max. at 227, 258, 269, 280 $\text{m}\mu$ ($E_{1\text{cm}}^{1\%}$ 450, 900, 1050, 990) and infra-red absorption bands (cm^{-1}) at, *inter alia*, 3302 s, 1741 w, 1668 ms, 1629 ms, 1570 m, 1039 w, 992 s, 969 m, 937 w, 929 w.

The light petroleum mother-liquors were then chromatographed on alumina, development being effected by acetone-light petroleum (b. p. 40—60°). A fast-running yellow band was followed by a wide green band. Eight fractions were taken, falling into three groups (the following abbreviations are used: s = strong, m = medium, w = weak, ms = medium-strong, i = inflexion. 1% refers to the percentage of *Tenebrio molitor* immobilised 24 hr. after topical application of a 4% w/v solution in acetone): (a) Fraction 1; a yellow liquid (0.21 g.) depositing a few hair-like crystals at 0°, insecticidally inactive (*Tenebrio molitor*), with no pungent taste. Ultra-violet absorption max. at 227, 251, 258, 285 $\text{m}\mu$ ($E_{1\text{cm}}^{1\%}$ 400, 160, 160, 210); infra-red absorption bands (cm^{-1}) at 1735 m, 1616 w, 1040 s, 993 m, 948 i, 935 m. (no absorption at 3200—3400 cm^{-1}). (b) Fractions 2—5 (5.0 g.), amber or yellow green, waxy or partly crystalline at 0°, unstable in air, highly insecticidally active (I% 92—96%), and possessing a burning taste and sialogogue effect. The following data relate to fraction 4 (1.52 g.): light absorption max. at 227, 269, 280 $\text{m}\mu$ ($E_{1\text{cm}}^{1\%}$ 400, 1050, 780); infra-red absorption bands (cm^{-1}) at 3302 s, 1672 ms, 1632 s, 1553 m, 1039 w, 992 s, 969 i, 922 w. (c) Fractions 6—8 (3.15 g.), yellow green, depositing fine crystals at 0°, insecticidally inactive, burning taste absent though bitter. The following data relate to fraction 6 (2.45 g.): light absorption max.

at 227, 251, 270, 280 μ ($E_{1\text{cm}}^{1\%}$ 520, 160, 520, 660); infra-red absorption bands (cm^{-1}) at 3374 (w and broad), 1655 ms, 1612 ms, 1511 ms, 1038 s, 1001 w, 976 m, 932 m.

Herclavin has light absorption max. at 223 and 280 μ (ϵ 21,500 and 22,700) and infra-red absorption bands (cm^{-1}) at *inter al.*, 1655 s, 1610 s, 1576 w, 1509 m, 1036 i, 1027 s, 991 ms, 982 s (no absorption at 3200—3400 cm^{-1}). Asarinin has light absorption max. at 236 and 286 μ (ϵ 9600 and 9600) and infra-red absorption bands (cm^{-1}) at *inter al.*, 1255 s, 1247 m, 1225 w, 1192 m, 1179 m, 1100 m, 1074 s, 1050 w, 1036 s, 998 w, 986 w, 968 w, 959 m, 936 m, 928 s, 892 w, 881 m, 869 w, 836 w, 824 m, 809 m, 795 s, 786 w, 737 m (no absorption at 3200—3400 cm^{-1}). Asarinin and herclavin are insecticidally inactive.

neoHerculin has been isolated from two specimens of bark, but a third gave little or none, although asarinin and herclavin were present as usual.

neoHerculin.—The crude material (above) formed *neoherculin* as colourless needles, m. p. 69—70°, from light petroleum (b. p. 40—60°) at 0° but tended to separate as a bulky gel which gave a hard pad when filtered off (Found: C, 77.3; H, 10.2; N, 5.7. $\text{C}_{16}\text{H}_{25}\text{ON}$ requires C, 77.65; H, 10.2; N, 5.65%). A Rast molecular-weight determination was not possible as the sample decomposed when fused with camphor. *neoHerculin* is soluble in most organic solvents: in air at 25° it becomes yellow and sticky, then forming a hard brown varnish. For light absorption data see Table. The infra-red absorption was closely similar to that of the slightly less pure material m. p. 63—65° mentioned in Part I, where the spectrum is given.

Hydrogenation of neoHerculin.—On microhydrogenation in glacial acetic acid with Adams catalyst *neoherculin* absorbed hydrogen equivalent to 3.75 (3.8, 3.75, 3.65) double bonds. In a preparative experiment, ethyl acetate was used as solvent (3.6 mols. absorbed). After separation of the catalyst and evaporation, *N*-isobutyldodecanamide, m. p. 53—54°, was isolated, undepressed by admixture with an authentic specimen (m. p. 53°). The saturated amide (11.6 mg.) was hydrolysed at 100° with concentrated hydrochloric acid (0.2 ml.) and ethanol (0.8 ml.) for 48 hr. The product was added to excess of 2*N*-sodium hydroxide and refluxed for 1 hr. Acidification and isolation in the usual way yielded the crude acid (4.8 mg.). This was chromatographed (Howard and Martin's technique, *Biochem. J.*, 1950, 46, 532; cf. Part IV). The acid was eluted in the characteristic position for dodecanoic acid and contained less than 1.5% of decanoic acid. No acids of greater chain length were detected.

Action of Maleic Anhydride.—*neoHerculin* (25 mg.) was sealed with maleic anhydride (10 mg.) and benzene (0.2 ml.) in a nitrogen atmosphere. After heating periods of 2, 4 and 10 hr. at 100° no crystallisation could be induced by cooling. No crystals were obtained after 14 days at 0° and evaporation of the solvent gave only a resin.

Acid Hydrolysis of neoHerculin.—The amide (46 mg.) was heated at 120° for 60 hr. in a sealed tube with concentrated hydrochloric acid (0.3 ml.) and ethanol (0.5 ml.). The product was extracted with ether. Evaporation of the aqueous phase and extraction with ethyl acetate gave *isobutylamine* hydrochloride, m. p. 170°, in plates or needles (undepressed by authentic material, m. p. 173—174°). The chloroplatinate, prepared in ethanol, formed plates, m. p. 235° (decomp.); authentic *isobutylamine* chloroplatinate had m. p. 240°; admixture with the naturally derived specimen gave m. p. 235—240° but m. p.s are somewhat dependent on rate of heating.

Ozonolysis of neoHerculin.—Ozonised oxygen was passed through the amide (150 mg.), dissolved in glacial acetic acid (4 ml.), for 80 min. Zinc dust (1 g.) and water (40 ml.) were added and *ca.* 5 ml. of the mixture distilled into freshly prepared Brady's reagent. After 48 hr. the crude 2:4-dinitrophenylhydrazone was collected and boiled with benzene (100 ml.), cooled, and filtered. The benzene extract was chromatographed on alumina (10" by 1") and the faster-running zone eluted. Evaporation gave a yellow solid (94 mg.) which, when crystallised once from ethanol, had m. p. 159° (45 mg.). After two further crystallisations it formed shining yellow plates, m. p. 165° undepressed when mixed with acetaldehyde 2:4-dinitrophenylhydrazone, m. p. 167°. Admixture with formaldehyde or propaldehyde 2:4-dinitrophenylhydrazone caused large depressions. A blank ozonisation experiment resulted in no precipitate being formed with Brady's reagent.

The author is indebted to Dr. M. Elliott and Mr. P. Needham for the insecticidal data relating to *Tenebrio molitor* mentioned in this and the succeeding three Parts. This arrangement was made through the kindness of Dr. C. Potter (Department of Insecticides and Fungicides, Rothamsted Experimental Station).