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# CHEMICAL CONSTITUENTS OF THE BRIGHT ORANGE APHID, APHIS NERII FONSCOLOMBE *1

## I. NERIAPHIN AND 6-HYDROXYMUSIZIN 8-O-β-D-GLUCOSIDE

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The unusually colored bright orange aphid Aphis nerii (Homoptera: Aphididae) is frequently encountered in the warmer parts of the world feeding on the cardenolide-rich plants Nerium oleander (L.) (Apocynaceae) and Asclepias curassavica (L.) (Asclepiadaceae). *3 We have discovered large colonies of the species on A. curassavica growing in moist fallow fields near Rio de Janeiro; a total of 133 g. of fresh aphids (33 g. dry weight) has been extracted to date, permitting us to write a preliminary report on the chemical composition including structures of the major constituents.

The live aphids were removed from stalks and leaves by scraping or tapping and directly crushed under acetone. *4 Extraction was completed with further portions of acetone and ethyl acetate, and the solid residue from evaporation of the combined extracts (50% of the dry weight of the aphids) added directly to Florisil in ethyl acetate. The solvent front carried a mixture of triglycerides (23% of the dried aphid) contaminated with about 0.3% of a carotenoid

*1 Presented in part at the second Jamaica Natural Products Symposium, Kingston, January 1-4, 1968. This joint project grew out of the chance discovery of A. nerii on Asclepias curassavica growing near the U.W.I. chemistry building, by KB and UW, during the first Symposium, Jan. 3-6, 1966. The aphid species was kindly identified by Mr. Thomas Farr of the Institute of Jamaica, Kingston.

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*3 A small colony was also observed by KB in June 1968 on wild Oxypetalum (Asclepiadaceae) in northern Espírito Santo. Additional food-plants reported in Brazil include Araujia sericifera (Asclepiadaceae), Solanum nigrum (Solanaceae), Jasminum (Oleaceae), and Adiantum (Polypodiaceae) (1).

*4 Extraction of dead or dried aphids gave much reduced yields of glycosides.

mixture having UV absorption essentially identical with that of β-carotene. The principal triglyceride component (crystallized directly from the mixture in acetone) showed an ultraviolet maximum at 262 nm and was identical in every respect with a synthesized sample of 2-trans,trans-sorbo-1,3-dipalmitin, m.p. 62°. Although this is a new compound, the corresponding 2-sorbo-1,3-dimyristin has been isolated from a variety of temperate-zone aphid species (2). The detailed composition of the triglyceride mixture was ascertained by mass spectrometry and will be the subject of a future paper (3).

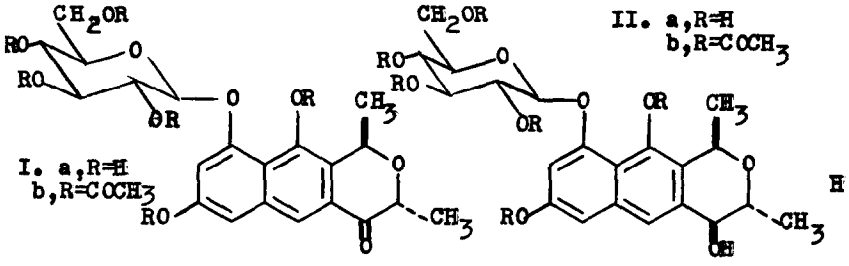
Gradient elution of the Florisil column with increasing percentages of methanol in ethyl acetate gave, in order: a narrow band of a red pigment, as yet uninvestigated; a wide band of the principal yellow pigment, neriaphin (Ia) *5 (4% of the dried insect); a partially overlapped band (purifiable by direct crystallization or rechromatography) of a colorless, non-fluorescent glycoside IIIa (1.2%); and narrow bands of two light yellow glycosides with exceedingly strong yellow-white fluorescence (0.2% and 0.3% respectively). Although a wealth of information has been accumulated on these last two compounds, which are very widespread in aphids (4), their most unusual structures cannot as yet be drawn with certainty; several structures compatible with available data are presently being synthesized from neriaphin.

The remainder of the total extract, mostly brown solid presently under investigation, could be eluted from the Florisil column with methanol.

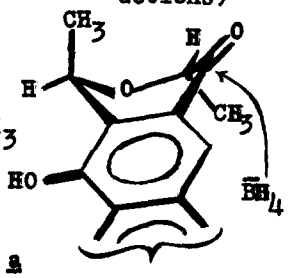
Crystallization of chromatographically purified neriaphin from methanol-isopropyl ether gave bright yellow needles, m.p. 213-215° dec., $C_{21}H_{24}O_{10}$. The IR spectrum (1688, 1630, 1582 $cm.^{-1}$) and the UV spectrum (see Table I) suggested the presence of a polyhydroxy-naphthalene ketone. Acid hydrolysis gave D-glucose (identified as its pentabenzoate and phenylosazone) and a very unstable aglycone whose peracetate had a UV spectrum (Table I) similar to that of β-acetonaphthone (5). The mass spectrum of neriaphin showed only peaks for the aglycone ($M^+$ at m/e 274.085 = $C_{15}H_{14}O_5$; strong peaks for loss of $CH_3$, $CH_3CHO$, $CH_3CH_2CO$, and $CH_3CH_2CO + CH_3$). The NMR spectrum (Table I) showed

*5 Although neriaphin is characteristically the major pigment of A. nerii, it has also been found as a minor constituent of other aphids (Periphyllus testudinaceus (Fernie) and Dactynotus spp.; H.J. Banks and D.W. Cameron).

three aromatic protons (one isolated, two in meta-disposition), two isolated and deshielded $CH-CH_3$ groupings, and the seven protons of a $\beta$-D-glucoside. In the spectrum of the acetylated aglycone (Table I), the seven glucose protons were missing and three aromatic O-acetyl groups appeared. From these facts, structure Ia was considered as probable for neriaphin. The peracetate Ib showed the expected peaks in the NMR spectrum (Table I), and a clear and diagnostic mass spectrum, revealing all the predictable ions from fragmentation of the intact molecule and of its sugar (6) and aglycone moieties.




(ring in boat to eliminate 1,3-diaxial interactions)



a

(peri-repulsion forces $\beta$-methyl to adopt axial conformation)

Reduction of neriaphin with sodium borohydride in cold ethanol gave totally stereospecific attack (a) (7); the single product obtained was shown to be identical with Glucoside B (IIa; Table I), previously known from reductive cleavage of the protoaphins (8), by IR, UV, and NMR spectra, thin layer chromatography and fluorescence, optical rotation, melting point, and mixture melting point, including of the respective peracetates (IIb). Thus, structure and configuration Ia can be considered as established for the major pigment of Aphis nerii. A small amount of Glucoside B (IIa) could also be isolated from the mother liqours of neriaphin.

The colorless glycoside IIIa crystallized from ethyl acetate in flattened needles, m.p. 214-215°. The IR (1655 $cm.^{-1}$, shifted to 1705 $cm.^{-1}$ in the peracetate IIIb) and UV (see Table I) spectra suggested the presence of an ortho-chelated polyhydroxy-$\beta$-acetonaphthone. The NMR spectra of the glycoside and its peracetate (Table I) showed the same $\beta$-D-glucoside protons and three aromatic protons as in neriaphin; however, the signal for the isolated aromatic proton was at much higher field and broadened by coupling with an aromatic methyl group whose signal was similarly split at the peak. A very narrow peak

TABLE I

NMR and UV Spectra of Glycosidic Compounds from *A. nerii* and Derivatives

| Compound | UV maxima (EtOH) in nm and (log ε) | NMR solvent | Aromatic protons | Aliphatic protons Glucose | CH | $CH_3$ | Acetate protons Glucose | Acetate protons Aglycone |
|---|---|---|---|---|---|---|---|---|
| Neriaphin (Ia) | 223(4.31),259(4.34) 306(3.78),412(3.47) infl. 233(4.27) 274(4.27) | acetone-$d_6$ | 7.83s 7.29$d_1$ 7.07$d_1$ | 5.22$d_2$ 3.93$d_3$ 3.65$m_1$ | 5.40q 4.72q | 1.66$d_2$ 1.41$d_2$ | | |
| | | pyridine | * | 5.81$d_2$ 4.40$m_2$ | 5.86q 4.93q | 1.71$d_2$ 1.57$d_2$ | | |
| 6-Hydroxymusizin 8-O-β-D-glucoside (IIIa) | 236(4.47) 268(4.25) 332(3.72) 345(3.72) | $CD_3OD$ | 6.94$s_1$ 7.00$d_1$ 6.73$d_1$ | 5.12$d_2$ 3.90$d_3$ 3.55$m_1$ | - | 2.60s 2.30$s_1$ | | |
| | | pyridine | * | 5.77$d_2$ 4.38$m_2$ | - | 2.70s 2.43$s_1$ | | |
| Glucoside B (IIa) | 238(4.68),246(4.62) 284(3.68),296(3.80) 309(3.80),338(3.58),351(3.60) | pyridine | * | 5.90$d_2$ 4.35$m_2$ | ~4.2q$d_3$ 5.68q 4.81$d_3$ | 1.70$d_2$ 1.58$d_2$ | | |
| 6-Hydroxymusizin (IVa) | 235(4.24) 273(4.28) 398(3.75) | $CH_2Cl_2$-MeOH | 6.84$s_1$ 6.52$d_1$ 6.41$d_1$ | - | - | 2.60s 2.47$s_1$ | | |
| Neriaphin peracetate (Ib) | 219.5(4.25),260 (4.50), 287(3.64) 296.5(3.70),367(3.33) | $CDCl_3$ | 8.42s 7.50$d_1$ 7.04$d_1$ | 5.27$m_3$ 4.23$m_3$ | 5.22q 4.65q | 1.65$d_2$ 1.49$d_2$ | 2.09s,2.06s 2.04s,1.98s | 2.43s 2.35s |
| 6-Hydroxymusizin 8-O-β-D-glucoside peracetate (IIIb) | 233(4.32) 295(3.86) | $CCl_4$ | 7.65$s_1$ 7.45$d_1$ 7.13$d_1$ | 5.35$m_3$ 4.28$m_3$ | - | 2.46s 2.36$s_1$ | 2.10s,2.04s 2.03s, 2.00s | 2.34s 2.32s |
| Glucoside B peracetate (IIb) | 237(4.70) 300(3.70) 335(3.34) | $CCl_4$ | 7.46s 7.20$d_1$ 6.87$d_1$ | 5.18$m_3$ 4.14$m_3$ | 5.72$d_2$ 5.22q 4.02q$d_2$ | 1.57$d_2$ 1.20$d_2$ | 2.03s,2.00s 1.98s,1.93s | 2.33s 2.21s 2.12s |
| Neriaphigenin triacetate | 221(4.32),254(4.52) 281(3.84),293(3.85) 352(3.33),364(3.33) | $CCl_4$ | 8.74s 8.00$d_1$ 7.32$d_1$ | - | 5.34q 4.74q | 1.62$d_2$ 1.47$d_2$ | - | 2.41s 2.35s 2.33s |
| 6-Hydroxymusizin triacetate (IVb) | 229(4.69) 289(3.78) | $CCl_4$ | 7.72$s_1$ 7.68$d_1$ 7.17$d_1$ | - | - | 2.49s 2.39$s_1$ | | 2.33s 2.33s 2.26s |

EXPLANATION

All NMR values in δ downfield from TMS

s = sharp singlet

$s_1$ = broadened singlet, J=0.7

$d_1$ = doublet, J=2

$d_2$ = doublet, J=6-7

$d_3$ = doublet, J=9

q = quartet, J=6-7

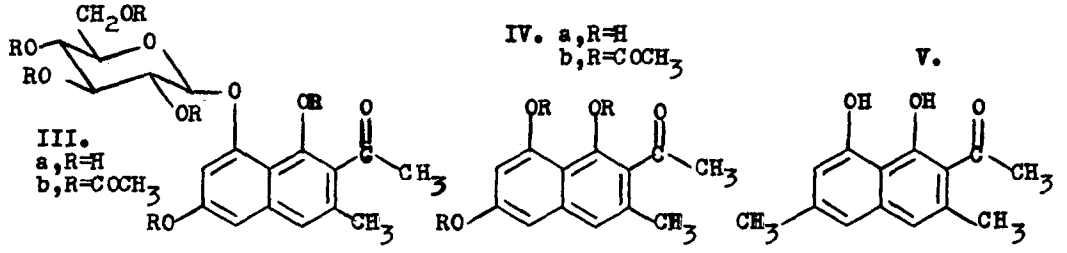
$m_1$ = 5H, broadened single peak showing fine structure

$m_2$ = 6H, high single peak with long tail upfield

$m_3$ = 5H (~5), 2H (~4) broad single peaks

* peak under solvent

for an acetophenone-type methyl group was also evident. The mass spectrum revealed only the molecular ion for the aglycone (m/e 232) and an equally strong peak for loss of a methyl group (m/e 217.049 = $C_{12}H_9O_4$; $m^*$ at 203). Thus, structure IIIa = 2-acetyl-3-methyl-1,6,8-trihydroxynaphthalene 8-O-β-D-glucoside (=6-hydroxymusizin glucoside (9)) seemed probable for the colorless glycoside. Acid hydrolysis gave D-glucose (as pentabenzoate and phenylosazone) and a crystalline but unstable yellow aglycone IVa with a UV spectrum (Table I) similar to that of musizin, whose 8-mono-ethers are invariably colorless (9). The peracetyl-aglycone IVb had a clear NMR spectrum substantiating all elements of its structure (Table I); its UV spectrum (and also that of the peracetyl-glucoside IIIb) was identical with that of naphthalene, showing that the carbonyl group is forced out of conjugation with the ring through steric repulsion by the 1-acetoxy group.




The aglycone IVa is one of the few known natural products formally derivable by direct cyclization (with elimination of water and $CO_2$, but without subsequent oxidation or reduction steps) of a hypothetical heptaketide (10), and is also very close to the compound isolated by Collie from condensation of two moles of hepta-2,4,6-trione (V) (11). Its unambiguous synthesis from one of Collie's intermediates is presently underway.

The presence of the unusual polyketide glucosides I, II, and III in Aphis nerii along with equally unusual triglycerides (3) suggests the operation of singular biochemical mechanisms within this species (2). It seems possible that some or all of these compounds are biosynthesized by microphytosymbionts within the mycetones of the insect's cells. Furthermore, the apparent absence of any accumulation of cardenolides in A. nerii feeding on Asclepias implies that the insect is not truly aposematic (warningly colored) or distasteful.

This conclusion is supported by the impressive number of normal aphid predators observed feeding on *A. nerii* in the fields near Rio.

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