The Secretion of the Myriapod *Polydesmus collaris collaris* (Koch)

Diplopod Myriapoda include a number of species which are remarkable for the repellent secretions they can emit. These secretions, produced by glands provided with reservoir whose ducts discharge into small openings located at the surface of the dorsal sclerites, are generally emitted for defensive and offensive purposes when the myriapod is disturbed. Some tropical species are known in the literature for their capacity of ejecting the poison load to distances of up to 80 cm[1], and cases have been reported of cutaneous and ocular lesions being produced by this secretion, even in man. Several species of Diplopoda have been investigated from the point of view of the chemical composition of the defensive secretion, and the Table summarizes the data at present available.

Considering the two different systematical groups in which the examined species may be classified, a relationship can be noticed between morphological group and chemical composition of the defensive secretion produced. Thus, the flat, 'polydesmiform' species 1–9 emit repellent secretions containing hydrocyanic acid and free from quinones, which are characteristic for the secretions of the 'iuliform' species 10–19. This correspondence between morphological and chemical characteristics, which has been observed also for other groups of animals, appears to be significant, and is indicative of particular, complex lines of evolution.

It was therefore interesting to extend the chemical investigation to other species of Diplopoda, and in this paper *Polydesmus collaris collaris* (Koch) was studied, a myriapod of frequent occurrence in Italy in the hilly and mountainous districts of the Apennines, which produces a secretion of strong, bitter almond-like smell.

In order to obtain the secretion as pure as possible, the living myriapod was stimulated by exerting with a pincette a gentle sidelong squeeze. The water-clear exudate was collected by rubbing the millipede along the inner surface of a glass test tube, and then removed by washing with pure ether. From 9000 individuals of both sexes, treated as described, about 4000 ml of a very dilute ether solution of the secretion were obtained. After removal of most of the solvent by cautious distillation in an efficient column, the enriched ether solution (A) (about 25 ml) was washed with 5% aqueous sodium hydroxide; the organic neutral layer (B), separated from the alkaline aqueous layer (C), gave, after evaporation of the ether, a small oily residue (about 0.1 ml) of strong and pleasant aromatic smell. A small sample of the oil, distilled up to 244° at ordinary pressure in a glass capillary tube, showed evolution of gas; and gave, besides a high boiling residue, a droplet of a colourless liquid of strong bitter almond smell, which was identified as *benzaldehyde* (UV- and IR-spectra, formation of crystals of benzoic acid on exposure to the air). The bulk of the oily residue was then distilled *in vacuo*; after elimination of the benzaldehyde the whole was collected as a single fraction b.p. 180–200°/0.9 mm. Gas chromatographic analysis of the liquid (Aerograph

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Species	References	1,4-Benzoquinone	2-Methyl-1,4-benzoquinone	2-Methyl-3-methoxy-1,4-benzoquinone	Quinones (not characterized)	Hydrocyanic acid	Benzaldehyde	Glucoside of *p*-isopropil mandelic nitrile	Sugars
Order Proterospermophora (*Polydesmoidea*)									
1. *Pseudopolydesmus serratus* Say	6					+			
2. *Orthomorpha gracilis* Koch[a]	3,4					+	+		
3. *Rhysodesmus vicinus* Sauss[b]	2					+		+	
4. *Fontaria virginiensis* Drury[c]	5					+			
5. *Pachydesmus crassicutis* Wood	8					+	+		+
6. *Apheloria corrugata* Wood	6					+	+		
7. *Nannaria sp.* (not identified)	6					+			
8. *Cherokia georgiana* Bollman	6					+			
9. *Oxydus gracilis* Koch	6					+			
Order Opistospermophora (*Iuliformia*)									
10. *Archiulus* (*Schizophyllum*) *sabulosos* L.	9		+	+					
11. *Schizophyllum mediterraneum* (= *Iulus terrestris* L.)	4,10	+							
12. *Spirostreptus virgator* Silv.	11		+		+				
13. *Spirostreptus castaneus* Attems	11	+							
14. *Pachybolus laminatus* Cook	11		+						
15. *Chicobolus spinigerus* Wood	7		+	+					
16. *Floridobolus penneri* Causey	7		+	+					
17. *Narceus annularis* Raf	7		+	+					
18. *Narceus gordanus* Chamb	7		+	+					
19. *Trigoniulus lumbricinus* Gerst	7		+	+					

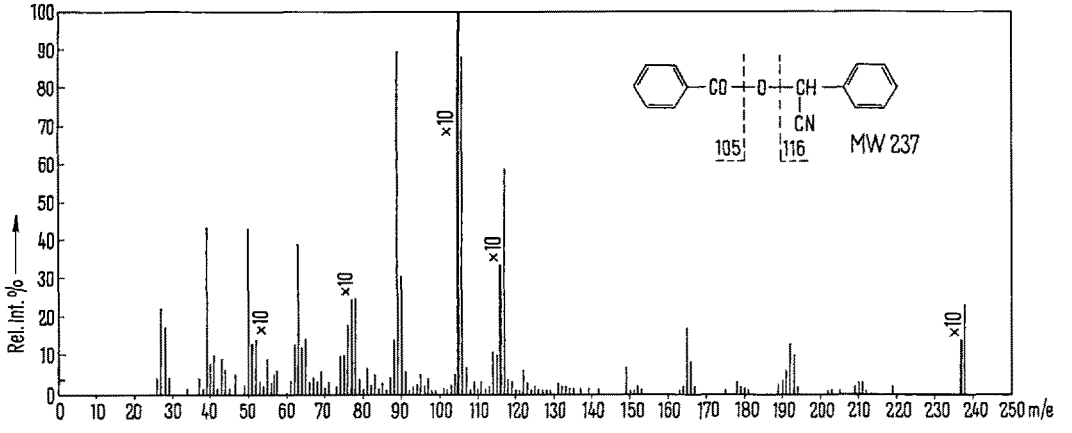
[a] Also reported in the literature as *Polydesmus* (*Fontaria*) *gracilis* Koch.
[b] Also reported in the literature as *Polydesmus* (*Fontaria*) *vicinus* L.
[c] Also reported in the literature as *Polydesmus virginiensis* Drury.

chromatograph, column of silicone S.E.-30, temperature 218°, 60 ml/min helium flow rate) gave after 25 min a single peak. The liquid product (chromatographic purity about 98%) corresponding to this peak has the IR-spectrum of a benzoate: C=O 1730 cm^{-1}; C—O 1242 and 1099 cm^{-1}; aromatic ring 1605, 1585 and 1495 cm^{-1}; aromatic CH 710 cm^{-1}. The UV-spectrum in ethanol (strong band at 232 mμ and benzenoid bands in the region 260–283 mμ) offers the general characteristics of benzoic acid esters but for a slight shift of 2–3 mμ in respect of the maxima of alkyl and benzyl benzoates.

Alkaline hydrolysis of a few mg of the product confirmed this assumption; pure benzoic acid was isolated, identified by its IR-spectrum and mixed melting point.

Since the extremely small quantity of the substance that was left after the tests described above would hardly have permitted a complete analysis, a reliable measure of the molecular weight, and the characterization of the hydroxylated moiety of the molecule, the investigation was continued by resorting to mass spectrography data.

The *mass spectrum* of the substance, obtained at 160° with an Atlas-Werke CH4 spectrometer, is reported in the Figure. The heaviest ion in the spectrum is 237, with isotopic ion 238. A spectrum at reduced electron accelerating voltage confirms that it corresponds to the molecular ion. The value of 237 found for the molecular weight suggests that the substance may contain nitrogen in odd number of atoms, most probably one. The fragmentation scheme reported below, in which only the most intense peaks have been considered, is in good agreement with that expected for a benzoate of molecular weight 237 containing a single nitrogen atom.



237^+ $C_6H_5COOR^+$ (p)
116^+ R^+ (p-C_6H_5COO)
115^+ (R-H)$^+$ (p-C_6H_5COOH)
105^+ $C_6H_5CO^+$ (p-OR)
89^+ (R-HCN)$^+$
77^+ $C_6H_5^+$

The peaks of lower mass are those characteristic for the benzene ring. The assignments postulated are supported by the interpretation of the metastable peaks observed:

$186.2 = 237^+ \rightarrow 210^+ + 27$
$141.5 = 192^+ \rightarrow 165^+ + 27$
$68.3 = 116^+ \rightarrow 89^+ + 27$
$56.8 = 237^+ \rightarrow 116^+ + 121$
$46.5 = 237^+ \rightarrow 105^+ + 132$

This scheme points to the existence of non-ionized fragments (not visible as such) of mass 27, 121 and 132. The dissociation of the substance, therefore, mostly follows the scheme:

$$237 + e \longrightarrow \begin{cases} \dashrightarrow 116^+ + 121 + 2\,e \\ \dashrightarrow 132 + 105^+ + 2\,e \end{cases}$$

These data would assign to the product the formula $C_{15}H_{11}O_2N = C_6H_5COO - C_8H_6N$.

Taking into account the mass spectrometry fragmentation pattern, the results of the chemical tests, and the finding of hydrocyanic acid in the alkaline washing (C) (see below), the most probable structure for the substance was (I) of *mandelic nitrile benzoate*[12]. Comparison with a

$$C_6H_5-\underset{\displaystyle CN}{\underset{|}{CH}}-OOCC_6H_5 \qquad (I)$$

synthetic sample of (I) confirmed that the assumption was correct. Racemic mandelic nitrile benzoate is a solid m.p. 62°, which has the same UV- and mass-spectra and the same retention time at the gas chromatography analysis as the natural product. Its IR-spectrum in the liquid state is identical to that of the product from natural source, but the spectrum of the solid contains some new bands, among them a very weak band at 2252 cm^{-1} assignable to the CN group. The liquid natural product should therefore represent one of the two optically active stereoisomers not yet isolated of (I) or a mixture of both not in equimolecular ratio; actually, a microdetermination of the specific rotation gave $[\alpha]_D^{19°} = +26.7 \pm 0.5°$. Since the benzoate of mandelic nitrile is not decomposed when heated at 120°–240°, whereas the crude neutral fraction of the secretion gives in the same conditions benzaldehyde and hydrocyanic acid, it may be inferred that mandelic nitrile as such is also present in the secretion; from this probably originates the hydrocyanic acid found in the alkaline washing. It must be mentioned that the benzoate of mandelic nitrile could also be isolated by direct steam distillation of the crude secretion.

Acid constituents of the secretion. A sample of the aqueous alkaline layer (C) was tested for the presence of CN^- ion; both the reaction with $FeSO_4$-$FeCl_3$ solution

[12] No band assignable to the CN group is found in the 2300–2000 cm^{-1} region of the IR-spectrum; the same has been observed for other liquid esters of α-hydroxynitriles. See L. J. BELLAMY, *The Infrared Spectra of Complex Molecules*, 2nd Ed. (1959), p. 266.

and HCl, and the spot test with Congo Red paper impregnated with $KHgCl_3$ solution[13] gave positive results. Also positive was the test with *o*-dinitrobenzene for the presence of benzoin, $C_6H_5COCH(OH)C_6H_5$[14], which must be considered an artefact originating from the simultaneous presence of benzaldehyde and HCN in alkaline milieu.

The bulk of the alkaline layer was acidified and repeatedly extracted with ether. The ether solution, dried on anhydrous sodium sulphate and reduced to small volume, was submitted to gas chromatographic analysis. It contained the following volatile fatty acids in the approximative ratios indicated: isovaleric acid (90%), formic acid (5.5%)[15], acetic acid (4%).

Riassunto. È stata studiata la secrezione odorosa che il Miriapode *Polidesmus collaris collaris* (Koch) emette quando è disturbato. Dalla frazione neutra è stato isolato e identificato un benzoato otticamente attivo del nitrile mandelico; benzaldeide e acido cianidrico sono stati pure ritrovati.

G. Casnati, G. Nencini[16], A. Quilico, M. Pavan[17], A. Ricca, and T. Salvatori[18]

Centro per lo Studio delle Sostanze Naturali del C.N.R., Politecnico di Milano (Italy), April 18, 1963.

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Gesteigerte Saugfrequenz von *Dactynotus jaceae* L. (*Homoptera, Aphididae*) bei Erhöhung des Aminosäurengehaltes in der Wirtspflanze

Die unterschiedliche Wirtseignung der einzelnen Entwicklungsstadien einer Pflanze für Blattläuse wird meist mit dem Gehalt des Phloemsaftes an löslichen Stickstoffverbindungen, Aminosäuren (AS) und Amiden, in Verbindung gebracht[1–3]; er ist in den bevorzugt befallenen stark wachsenden und seneszenten Trieben besonders hoch[4,5]. Dass bei ansteigender AS-Konzentration in der Pflanze das Wachstum und die Vermehrung von bereits saugenden Tieren gefördert werden, hat Mittler[5] bei *Tuberolachnus salignus* Gmelin gezeigt. Es blieb jedoch offen, ob die AS-Konzentration auch für das unterschiedliche Präferenzverhalten der nahrungssuchenden Blattläuse verantwortlich gemacht werden kann.

Mit apteren Virgines von *Dactynotus jaceae* s. str. wurden Alternativwahlversuche durchgeführt zwischen Paaren von *Centaurea jacea*-Sprossen, die sich in ihrem Gehalt an freien AS unterscheiden. Eine Erhöhung der AS-Konzentration – als Folge einer beschleunigten Seneszenz – ist bereits durch Abschneiden der Sprosse und etwa 2tägiges Einstellen in Wasser zu erreichen (vgl. Tabelle I: Kontrollpflanzen 0 h und 45 h). Abgesehen von der komplexen Natur solcher Alterungsprozesse machen jedoch dann die mechanischen Oberflächenveränderungen der relativ rasch austrocknenden Triebe einen vergleichenden Test mit frisch abgeschnittenen, weniger AS-reichen Sprossen unmöglich.

Stellen wir einen frischen Trieb (Knospen- oder Blütenstadium, 30 cm lang, untere Hälfte entblättert) 15 cm tief in eine 0,5%ige wässrige Lösung von l-Glutaminsäure (Glu), so steigt innerhalb von 15 h der Gesamtspiegel der freien AS im Stengel auf über das Doppelte des Ausgangswertes, während er sich bei der in Wasser eingestellten Kontrollpflanze noch kaum verändert (Tabelle I: 15 h). Beide Triebe sind nach der Behandlung gleich frisch und können nun im Wahlversuch einander gegenübergestellt werden.

Methodik der Wahlversuche: Je 2 von derselben Pflanze stammende, morphologisch gleichartige, aber verschieden vorbehandelte Triebe stehen mit ihren blattfreien Stielen in gemeinsamem Zylinder (4×9 cm) aus durchsichtigem Zelluloid; die Stielenden tauchen unter dem durchbohrten Zylinderboden in Gefässe mit Glu-Lösung bzw. Wasser. Besetzen der Zylinder mit 30 Virgines. Kontrolle der saugenden Tiere (Rüssel länger als 1 min eingebohrt!) in Abständen von 1–1¼ h. Nach jeder Kontrolle Abschütteln der Tiere von den Pflanzen.

Versuchsfolge:
0. Stunde: Triebe abschneiden,
1.–15. Stunde: 15 h Glu-(bzw. H_2O-)Behandlung,
16.–30. Stunde: 15 h Wahlversuch,
31.–45. Stunde: 15 h Glu-(bzw. H_2O-)Behandlung,
46.–55. Stunde: 10 h Wahlversuch.

Bestimmungen des AS-Gehaltes der im Wahlzylinder eingeschlossenen Stengelteile wurden vor und nach den beiden Glu- (bzw. H_2O-) Behandlungen durchgeführt.

Wie Tabelle I zeigt, unterscheiden sich die beiden zur Wahl gestellten Triebe in ihrer AS-Konzentration am meisten zur Zeit der ersten Wahlversuchsperiode (15–30 h nach Versuchsbeginn). Nach Tabelle II saugen während

Tabelle I. Gehalt an freien Aminosäuren bei Glu-behandelten und unbehandelten Trieben von *Centaurea jacea* zu verschiedenen Versuchszeiten. – Planimetrisch integrierte Flächen der Extinktionskurven aufsteigender, eindimensionaler AS-Chromatogramme. Mittelwerte aus 3 Extraktserien

h nach Versuchsbeginn	Glu-behandelt	H_2O-Kontrolle
0	–	17,3
15	42,6	18,7
30	49,6	21,6
45	62,7	36,5

Tabelle II. Saugverhalten von *D. jaceae* im Alternativwahlversuch mit Glu-behandelten und unbehandelten *Centaurea jacea*-Trieben. Ergebnisse aus 34 Einzelversuchen

Versuchszeit	15.–30. h		45.–55. h	
	Glu	H_2O	Glu	H_2O
Saugende Virgines absolut	3463	2954	1201	980
relativ	54,0%	46,0%	55,1%	44,9%
Kontrollen mit Befall von				
Glu > H_2O	259	(56,6%)	141	(48,6%)
Glu < H_2O	151	(33,0%)	99	(34,1%)
Glu = H_2O	48	(10,5%)	50	(17,2%)

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