Belg. J. Zool. — Volume 121 (1991) — issue 2 — pages 211-225 — Brussels 1991

(Manuscript received on 23 February 1991)

POTENTIAL TAXONOMIC APPLICATIONS OF H.P.L.C. ANALYSIS OF COCCOIDEA PIGMENTS (HOMOPTERA : STERNORHYNCHA)

by

JAN WOUTERS (1) and ANDRÉ VERHECKEN (2)

(1) Koninklijk Instituut voor het Kunstpatrimonium, Jubelpark, 1, B-1040 Brussels

(2) Koninklijk Belgisch Instituut voor Natuurwetenschappen, Vautierstraat, 29. B-1040 Brussels

SUMMARY

High Performance Liquid Chromatography (HPLC) was applied to separate and identify the constituents of the pigments of scale insects. We concentrated on the following problems : the classification of unsufficiently characterised Near-Eastern Kermesidae (*Kermes biblicus* and *K. palestiniensis*) ; the relationship between *K vermilio* and *K. ballotae*, living on the same plant species ; and the search for complementary evidence for a suggested identity of *Porphyrophora polonica* and *P. crithmi* (Margarodidae).

It was shown that the pigment composition of Near-Eastern Kermesidae might be helpful in further taxonomic studies. The differences between the pigments of *K. vermilio* and *K. ballotae* contradict their being only forms of the same species. The pigment composition of both *Porphyrophora* species studied is exactly the same.

Keywords : HPLC, pigments, chemotaxonomy, *Kermes, Porphyrophora.*

INTRODUCTION

Recently, the High Performance Liquid Chromatography (HPLC) analysis of pigments present in some species of scale insects of the superfamily Coccoidea has been worked out (WOUTERS and VERHECKEN, 1989). The main purpose of that study was to investigate the dye contents of the few species of *Kermes, Dactylopius, Porphyrophora* and *Kerria* which have been used historically for textile dyeing purposes. From this study it followed that dye analysis allows the species-level identification of the taxa studied, even when analysing only their alcoholic preserving fluids (WOUTERS, 1990). Most of this work was done on adult female insects. It was found that the pigments are present in the insect as free anthraquinone dyestuffs and/or as dyestuff precursors, probably anthraquinone glycosides.

During cited work, we came across a number of species for which the exact taxonomic status is not clearly defined. The present paper reports on the pigment content of these species. It is hoped that this new information may be useful to entomologists as an extra tool, possibly enabling them to gain a better understanding of the identity of these species and of the taxonomic relations between them.

MATERIALS AND METHODS

Samples

Specimens of a *Kermes* species collected at Kibbutz Eilon and nearby Adamit on the Lebanese border of Israel in June 1987, and identified as *Kermes biblicus* BODENHEIMER, 1926, by prof. Sternlicht, were obtained from dr. I. Ziderman (Israel Fiber Institute, Jerusalem). First instar larvae from this sample were identified by D. Matile-Ferrero (MNHN, Paris) as probably belonging to *K. palestiniensis* BALACHOWSKY, 1953, with the remark : « en l'absence d'éléments concrets nous permettant d'identifier correctement toutes les espèces de *Kermes* du bassin Méditerranéen, je ne tiens compte actuellement que des espèces accompagnées d'une description moderne et détaillée et notamment du travail de BALACHOWSKY (1953) ». This sample is here referred to as *K. biblicus,* not implying however the acceptance of this name as the valid one for the taxon.

Samples collected by the second author at Harbiye (southern Turkey, near Antakya) and Fethiye (western Turkey) in June 1989, were visually clearly different from each other because of the parallel black bands on the material from Fethiye. The latter might belong to the species provisionally named *Kermes virgatus* CARDON, 1990, cited for western and southwestern Turkey. Both our Harbiye and Fethiye samples were identified on the first instar larvae by D. Matile-Ferrero as *K. palestiniensis,* with the same restrictive remark as for the Israeli sample.

Kermes vermilio (PLANCHON, 1864) and *K. ilicis* (LINNÉ, 1758) were collected in Southern France by the second author (VERHECKEN and WOUTERS, 1990).

Specimens of *Kermes ballotae* SIGNORET, 1874, from the Pisa region (Italy) were donated by D. Matile-Ferrero.

Cysts and adult specimens of *Porphyrophora polonica* (LINNÉ, 1758), both before and after ovipositing, were given by E. Szymula (Jagellonean University of Krakow, Poland). The were found on the roots of *Scleranthus perennis* in the vicinity of Czestochova in May 1990.

Adult females of *Porphyrophora crithmi* (GOUX, 1938) from the South of France were donated by Dr. I. Foldi (MNHN, Paris) ; they were described as being fecondated.

Preparation of samples

It is preferred to analyse insects that were not killed by immersion in solvents and that were not put in a preserving liquid, to avoid any loss of pigments before

analysis. Some species (*e.g. Porphyrophora*) are treated in a delipidating solvent (methanol/chloroform, 2/1, v/v) which was shown to extract no pigment, to improve their subsequent aqueous extraction.

One to three air-dried insects are finely powdered and extracted in water/methanol (9/1, v/v) for various periods of time at room temperature or at the boil. The extracts are cleared under centrifugal force. Mild acid hydrolysis is performed to convert any acid-labile precursor to the actual pigment. Extracts are dried in a dessicator and the residues are dissolved in an appropriate volume of water/methanol (1/1, v/v) just before analysis. When the pigment content of these preparations is too low, they are absorbed on alum mordanted wool and recovered by acid hydrolysis (see Results).

Analytical procedure

High Performance Liquid Chromatography (HPLC) allows both separation and quantification of all the dye components present. Computerized diode-array detection makes it possible to analyse a sample in one single run at any wavelenght between 200 and 800 nm, i. e. the complete ultraviolet and visible spectrum. All the analytical data are stored on a computer hard-disk, from which they can be retrieved, printed and backed-up. A schematic representation of the analytical equipment is given by WOUTERS (1990). For the separation a column is used, 4.6 × 100 mm, filled with a reversed-phase adsorbent (Spherisorb ODS2, Biorad-RSL, Belgium). Typically 20 µl are injected and the column is then eluted with a concentration gradient of methanol in water, in the presence of phosphoric acid. Elution of one sample takes 30 minutes. At an eluent flow of 1.2 ml/min a system back pressure of 200 bar is generated. Further details are given in WOUTERS and VERHECKEN (1989).

RESULTS AND DISCUSSION

Selection of the way of presentation

The pigment content of scale insects known to have been used as dye sources in the past, usually varies between 1 and 20 % of the female body mass. Aqueous extracts of these species contain enough dye to be chromatographed as such (WOUTERS and VERHECKEN, 1989). At very low concentration however, individual dye components may be undetected in aqueous extracts and therefore other techniques must be applied.

Figure 1a gives the chromatogram, recorded at a wavelength of 275 nm, of an aqueous extract of *Kermes ilicis*. This wavelength ensures the highest possible sensitivity for most insect dyes (WOUTERS and VERHECKEN, 1989). Figures 1b and 1c depict the same analysis, presented however at 420 nm and 500 nm, in order to detect yellow and red dyes, respectively. It is clear that the dye content of the aqueous extract must be very low and that individual dye components may not be characterised properly.

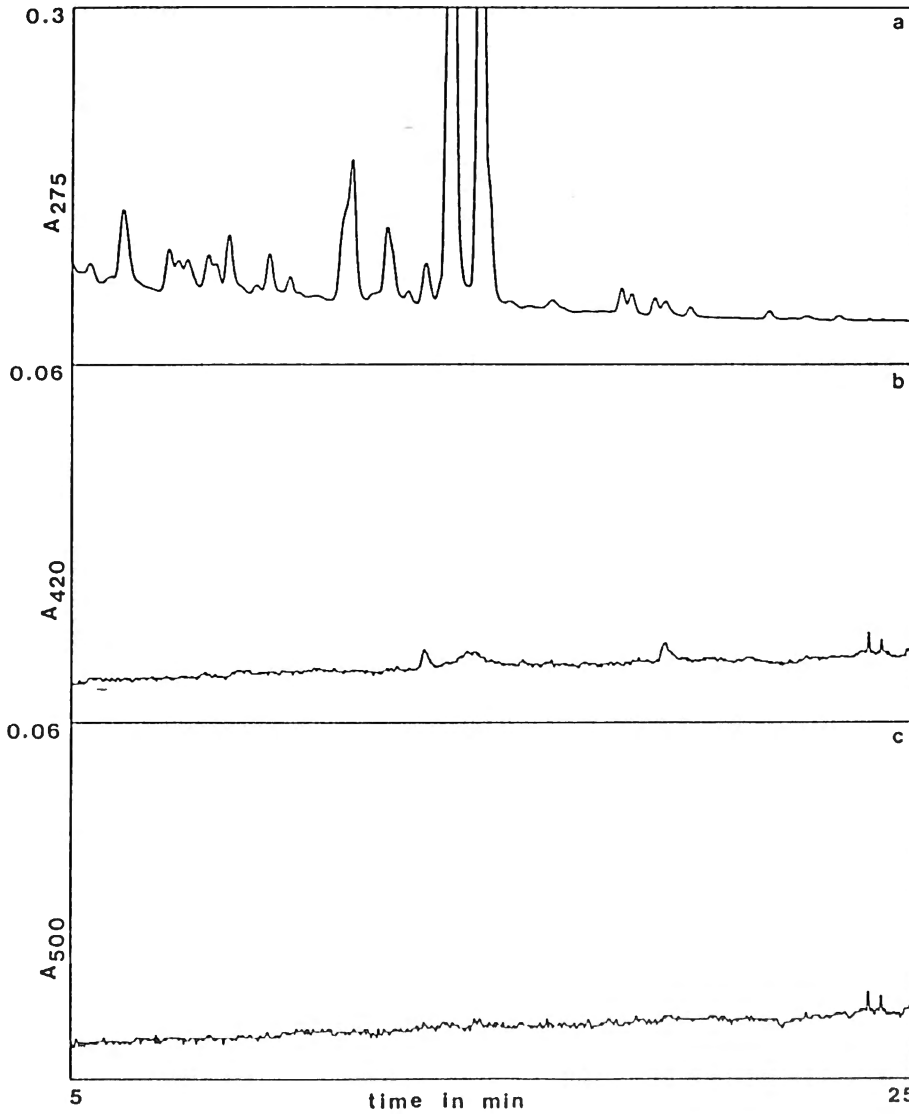

Fig. 1. — HPLC of *Kermes ilicis* pigments, monitored at (a) 275 nm, (b) 420 nm and (c) 500 nm. Aqueous extract.

In Figures 2a-c are given the chromatograms, taken at 275 nm, 420 nm and 500 nm, of an acidic hydrolysate of the aqueous extract, using exactly the same scaling as in Fig. 1a-c. It is obvious that the recovery of dyestuff is higher, probably due to hydrolysis of precursors.

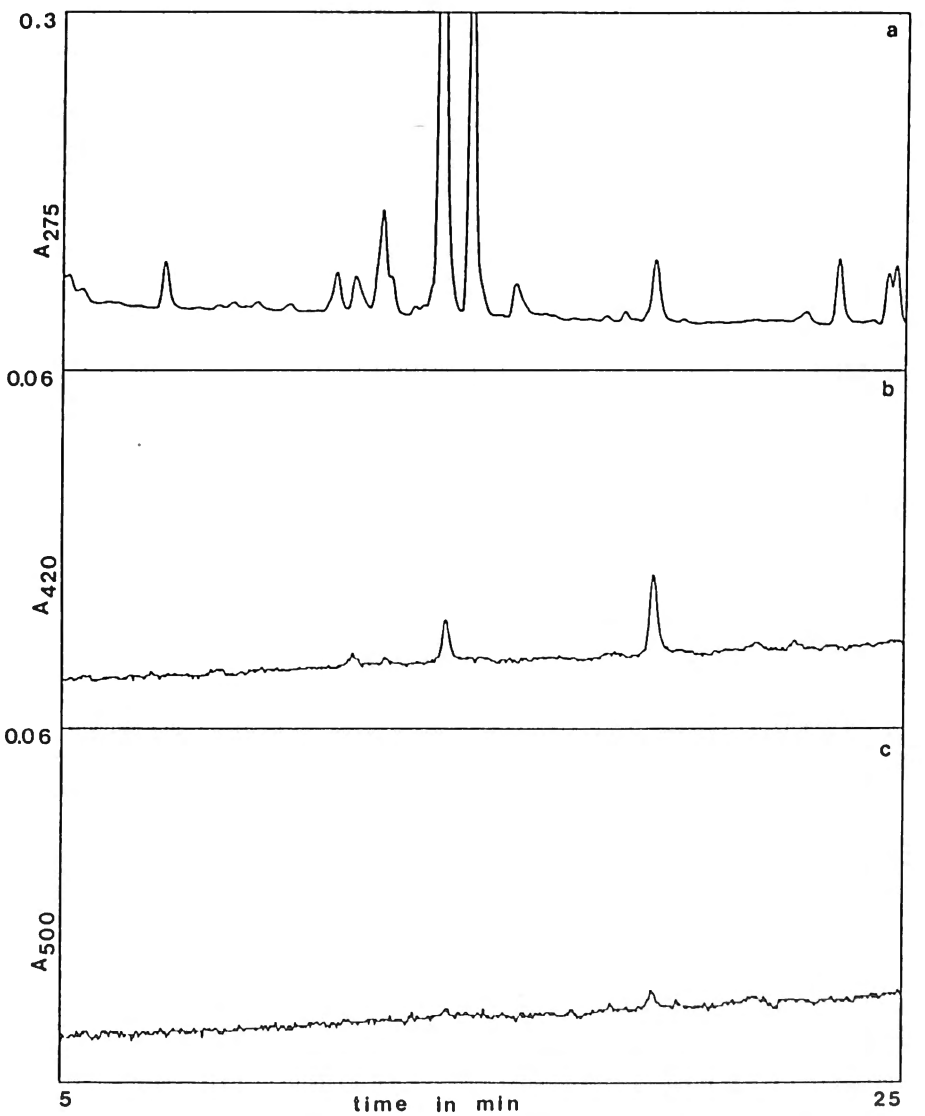

Fig. 2. — HPLC of *Kermes ilicis* pigments, monitored at (a) 275 nm, (b) 420 nm and (c) 500 nm. Acid hydrolysate of aqueous extract.

Figs 3a-c give the chromatograms, using exactly the same parameters as in Figs 1a-c and 2a-c, of a dye sample obtained from a wool yarn dyed with *K. ilicis*. The individual dye components are clearly recognisable now and their detection sensitivity is sufficient for spectral characterisation and quantification. The wool-dyeing experiment, originating from our previous work (WOUTERS and VERHECKEN,

1989a), increases the dye content of the samples to be analysed because the dyes are selectively taken up by the wool.

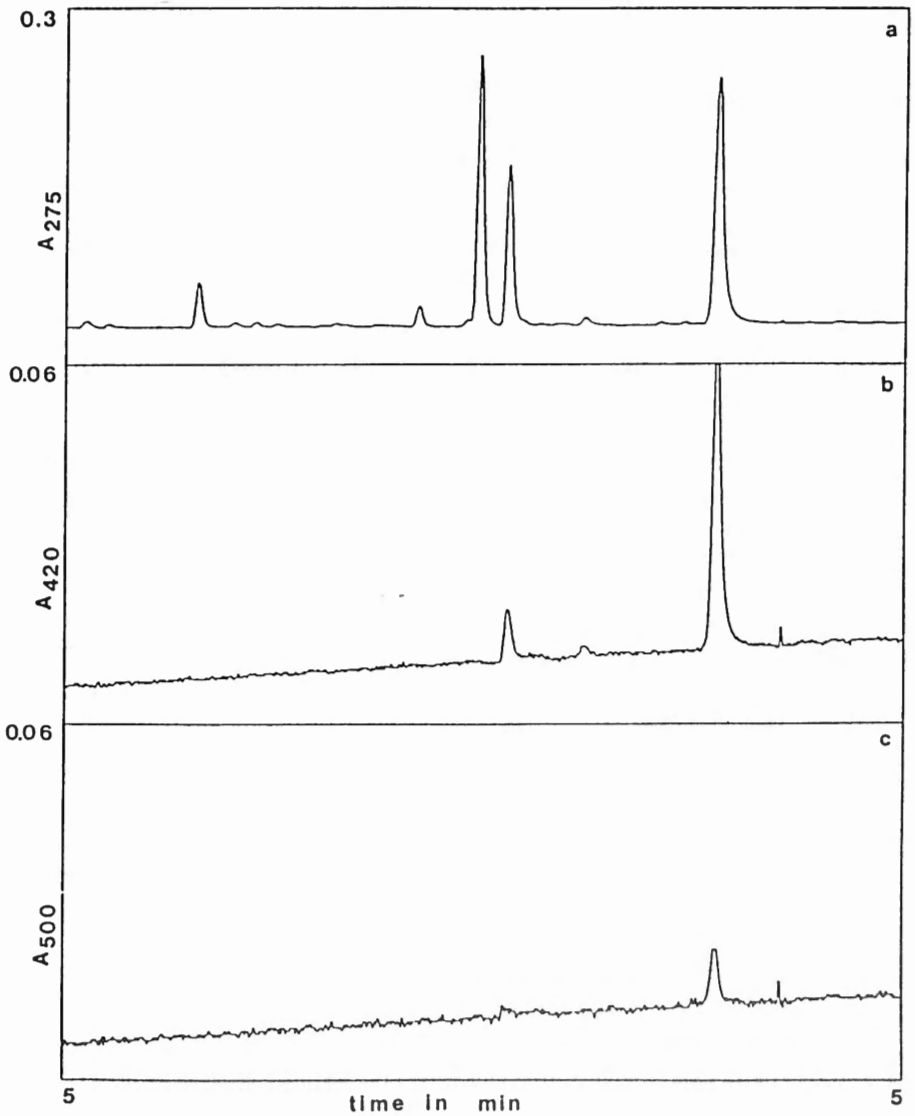

Fig. 3. — HPLC of *Kermes ilicis* pigments, monitored at (a) 275 nm, (b) 420 nm and (c) 500 nm. Pigments recovered from dyed wool.

Kermes biblicus, K. palestiniensis* and *K. ilicis

Kermes biblicus was described for adult specimens from Syria (actual Lebanon, see BEN-DOV and HARPAZ, 1985) ; BODENHEIMER (1931) later also introduced one species from Anatolia and two from Palestine. While reviewing the *Kermes* species of the eastern Mediterranean basin, BALACHOWSKY (1953) was unable to identify these species, since their description was only based on old adult females and not on first instar larvae, and Bodenheimer's type material could not be located. BALACHOWSKY (1953) thought it had disappeared (it is not in the Bodenheimer Collection, Hebrew University of Israel, Rehovot (BEN-DOV and HARPAZ, 1985), but it was reported recently to be in the American University in Beyruth (CARDON, 1990)) and therefore introduced three new species from Israel : *Kermes echinatus, K. spatulatus* and *K. palestiniensis*. He also stated that some of them might be identical with one of BODENHEIMER's species, and (BALACHOWSKY, 1950) that the description of *K. biblicus* might as well apply to *K. ilicis* LINNÉ.

STERNLICHT (1969) described another species for Israel : *Kermes bytinskii*. Study of specimens (not types ?) from Bodenheimer's collection (on loan from BMNH, London, and from prof. Harpaz (whereabouts not stated) led him to surmise, among others, that *K. palestiniensis* and *K. biblicus* are identical.

It is not clear why STERNLICHT (see samples) preferred the name *K. biblicus* to *K. palestiniensis* for the identification of the Israeli sample studied here since, while describing *K. bytinskii,* he implicitly considered BODENHEIMER's (1926, 1931) names to be *nomina nuda,* and in a later paper (STERNLICHT, 1980) he did not use the latter author's names for the two other Israeli species. Moreover, in 1980 he described the crawlers of *K. biblicus* as being red ; this was not the case in the specimens we received under that name. Preliminary tests did not show evidence of a red dye in this sample (VERHECKEN and WOUTERS, 1990). This is in contradiction with results obtained by TAYLOR (1988) by means of paper chromatography of a sample from the same source (but of the same identity ?) ; he suggests the presence of anthragallol and ceroalbolinic acid.

The pigments of the three species were concentrated on wool, since they were present in very low amounts. It is emphasised that the recovery of the dyes from the wool is always done in exactly the same way (WOUTERS and VERHECKEN, 1989a). The chromatograms obtained for *K. ilicis, K. biblicus, K. palestiniensis* (unstriped specimen from Harbiye) and *K. palestiniensis* (striped specimen from Fethiye) are given in Fig. 4a-d, respectively. The detection wavelength was 420 nm since no red pigments were shown to be present in a preliminary search. The identification of ceroalbolinic acid (caa) (GADGIL *et al.,* 1968) was made according to the presence of this peak in an acid extract of *Ceroplastes rubens* MASKELL, 1893 (WOUTERS, unpublished results), and to the data in BANKS (1977). Flavokermesic acid (fk) and kermesic acid (ka) were characterised previously (WOUTERS and VERHECKEN, 1989). The chemical structure of the three other peaks is unknown, therefore these are designated K1, K2 and K3 ; « K » from Kermesidae and the figure following the elution order. According to the chemical pathway presented by BROWN (1975), there should be a link between fk, desoxyerythrolaccin and both erythro- and

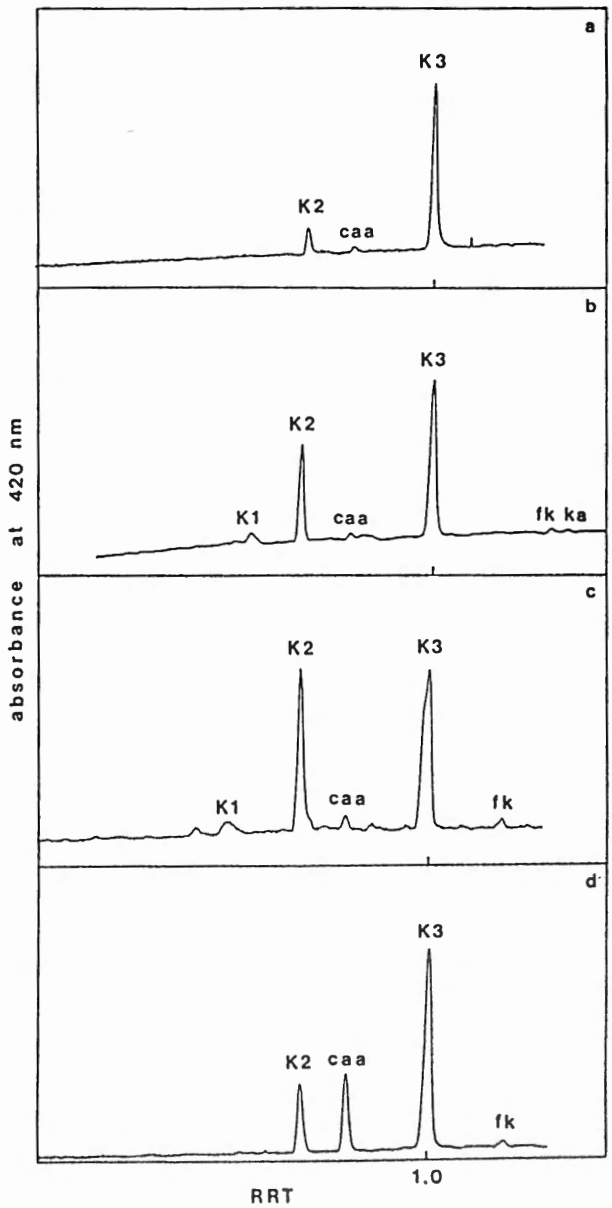

Fig. 4. — HPLC of Kermesidae pigments, recovered from dyed wool, monitored at 420 nm. (a) : *Kermes ilicis*; (b) : *Kermes biblicus*; (c) : *K. palestiniensis*, unstriped specimen; (d) : *K. palestiniensis*, striped specimen. The abbreviations refer to table 2. RRT : retention time, relative to K3 (RRT of K3 = 1.00). Small variations in RRT of fk and ka in (b) are due to modification of the elution program.

isoerythrolaccin. The identity of either of the latter products with K1, K2 or K3 could not be established because of the lack of the reference products, and since it was not clear whether or not the minor differences between the spectra we recorded and those given by THOMSON (1971) were due to the different solvents used. The two most related samples are *K. biblicus* and *K. palestiniensis* (unstriped).

The present findings support STERNLICHT's (1969) hypothesis regarding the possible identity of *K. palestiniensis* (in this case the unstriped specimen) and *K. biblicus*. Both *K. ilicis* and *K. palestiniensis* (striped) seem to be more divergent from the former and from each other : in *K. ilicis* only K3 is abundantly present ; in the striped *K. palestiniensis* important amounts of K2 and caa were also found, as well as some fk.

The occurrence of K1, K2 caa, K3 and fk in varying combinations suggests a close relationship of the species studied, and a particular position of this species group within the Kermesidae. It is very unlikely that scale insects with either of the four dye compositions found here may produce a red colour on wool. The only red component, ka, was found in *K. biblicus* but in so tiny an amount that its contribution to colour is negligible.

The two main components for *K. biblicus,* K2 and K3, may refer to the two spots obtained by paper chromatography of an acid insect extract (TAYLOR, 1988). However, the suggested identity of one of them with anthragallol may be ruled out definitely.

Kermes vermilio and *K. ballotae*

K. vermilio was described for the species living on the kermes oak *Quercus coccifera* and yielding the red dye historically used for dyeing textiles. *K. ballotae* is considered by BALACHOWSKY (1950a) as a form of *K. vermilio* living on *Quercus ilex* and *Q. suber*. He gives distinguishing features of adult females ; he states that their neonate larvae are strictly identical and consequently he understands both « forms » as belonging to a single polymorphous species. He never found *K. ballotae* on *Q. coccifera,* nor *K. vermilio* on *Q. ilex,* and concluded that the polymorphism and heterochromy are due to the habitat.

According to information obtained from D. Matile-Ferrero, both *K. vermilio* and *K. ballotae* are now reported to live on *Quercus coccifera* and on *Q. ilex* ; this changes the concept of their being only forms of the same species.

The detailed dye analysis of the females of *K. vermilio* was reported earlier (WOUTERS and VERHECKEN, 1989). In an extract of *K. vermilio,* three dyestuffs were obvious : kvI, fk and ka. Acid hydrolysis destroys kvI and generates both fk and ka. It was possible to reveal two components in kvI : kvIa and kvIb. Their spectrum suggests them to be precursors for fk and ka, respectively (WOUTERS and VERHECKEN, 1989). The relative abundances of fk and ka were 33/67 in the extract, 26/74 in the acid hydrolysate, and 14/86 in the sample prepared from dyed wool.

In the extract of *K. ballotae,* four dyestuff components were detected : kbaI, kbaII, fk and ka. Both kbaI and kbaII are hydrolysed in acid to fk. The relative

abundances of fk and ka in the extract were 63/37; after acid hydrolysis this changes into 75/25, and to 82/18 for a sample from dyed wool. Distinction with *K. vermilio* may be made either by analysis of the precursors or by considering the fk/ka ratio, the latter preferentially after acid hydrolysis.

The present data allowed us to reconsider the identity of a sample of crushed *« Kermes vermilio »* sent to us by Prof. Boyer (CNRS, Draguignan, France) several years ago. A HPLC analysis of it was already published (WOUTERS, 1985). It is now known that the large peak preceding ka in that chromatogram is fk, so that the identify of the sample must be *K. ballotae* rather than *K. vermilio*. Probably, this material had been identified according to its habitat, rather than on entomological features.

Porphyrophora polonica and *P. crithmi*

Porphyrophora polonica lives on roots of some plant species in Central and Eastern Europe. *P. crithmi* was described as a geographical subspecies of the North-African *Margarodes buxtoni* NEWSTEAD, 1917, for specimens living at Marseille on subterranean parts of *Crithmum maritimum*. It is judged to be identical, even at cytological level, with *P. polonica* by Dr. I. Foldi (CARDON, 1990), who requested us to check his findings by pigment analysis.

A chromatographic solution to this problem was not straighforward since it was known that the pigment composition of *P. polonica* varies with its development stage (WOUTERS and VERHECKEN, 1989) : at least seven components, including two acid-labile precursors, were present in varying ratios. The *P. crithmi* females sent by Dr. Foldi were described as follows in the accompanying letter : « 1 tube avec six

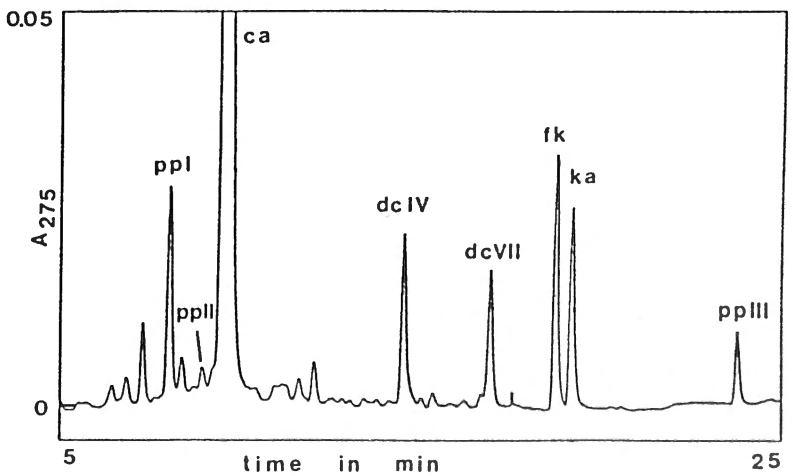

Fig. 5. — HPLC of an aqueous extract of *Porphyrophora crithmi* pigments (adult females, fecondated), monitored at 275 nm. Abbreviations refer to table 2.

TABLE 1

Dyestuff composition of *Porphyrophora polonica,* females (pp/f), in following stages : cyst (/c), before ovipositing (/bo), ovipositing (/o) ; and of *Porphyrophora crithmi,* females (pc/f), fecundated (/f). The addition /h points to a sample hydrolysed in acid. Figures represent integration values, taken at 275 mm ; figures between brackets represent relative ratios within the group of components considered.

Sample	ppI	ppII	ca	dcIV	dcVII	fk	ka	ppIII
pp/f/c	7.6	32	139 (86.9)	1.4	1.4	2.1 (1.3)	19 (11.9)	—
pp/f/c/h	—	—	123 (69.1)	3.2	2.7	9.1 (5.1)	46 (25.8)	—
pp/f/bo	13	29	258 (96.6)	4.6	2.2	2.3 (0.9)	6.7 (25)	—
pp/f/bo/h	—	—	226 (82.2)	5.7	5.0	13 (4.7)	36 (13.1)	0.4 (0.1)
pp/f/o	6.6	0.6	139 (97.9)	1.9	1.4	2.2 (1.5)	0.2 (0.2)	—
pp/f/o/h	—	—	114 (92.7)	3.0	2.5	7.6 (6.2)	1.3 (1.0)	0.3 (0.2)
pc/f/f	3.8	0.3	197 (95.2)	3.6	2.5	4.6 (2.2)	3.9 (1.9)	1.2 (0.6)
pc/f/f/h	—	—	177 (92.2)	4.6	4.2	8.4 (4.4)	4.5 (2.3)	2.3 (1.2)

femelles : la secrétion blanchâtre signifie qu'elles ont été fécondées ». Only recently we obtained several well identified lots of *P. polonica,* described as follows : « 1. female cysts ; 2. adult females before ovipositing ; 3. adult females, ovipositing ». The analytical results obtained from aqueous extracts and from acid hydrolysed extracts are given in Table 1. For clarity, one chromatogram is depicted also (Fig. 5), wherein each considered is designated by its abbreviation.

The *P. polonica* cysts were clearly distinct from the other samples in showing higher amounts in ka, especially after acid hydrolysis, and in being devoid of ppIII. A striking similarity occurs between *P. polonica* (females, ovipositing) and *P. crithmi* (females, fecundated) : in both cases fk prevails over ka, and the amounts of these latter compounds are relatively low compared to the other samples ; the presence of ppIII in the hydrolysed samples is also common to both samples ; and the ratio ppI/ppIII is the same in the preparations considered. Acid hydrolysis destroys ppI and ppII in all samples and an almost quantitative transition between either ppI and fk or ppII and ka can be seen, thus proving that ppI is the precursor of fk, and ppII the one of ka.

According to the pigment analyses, *P. polonica* is identical to *P. crithmi.* The entomological implication of the chemical data is not in contradiction with the distribution area of *P. polonica,* as described by KOSZTARAB and KOZÁR (1988) and by YASCHENKO (1990).

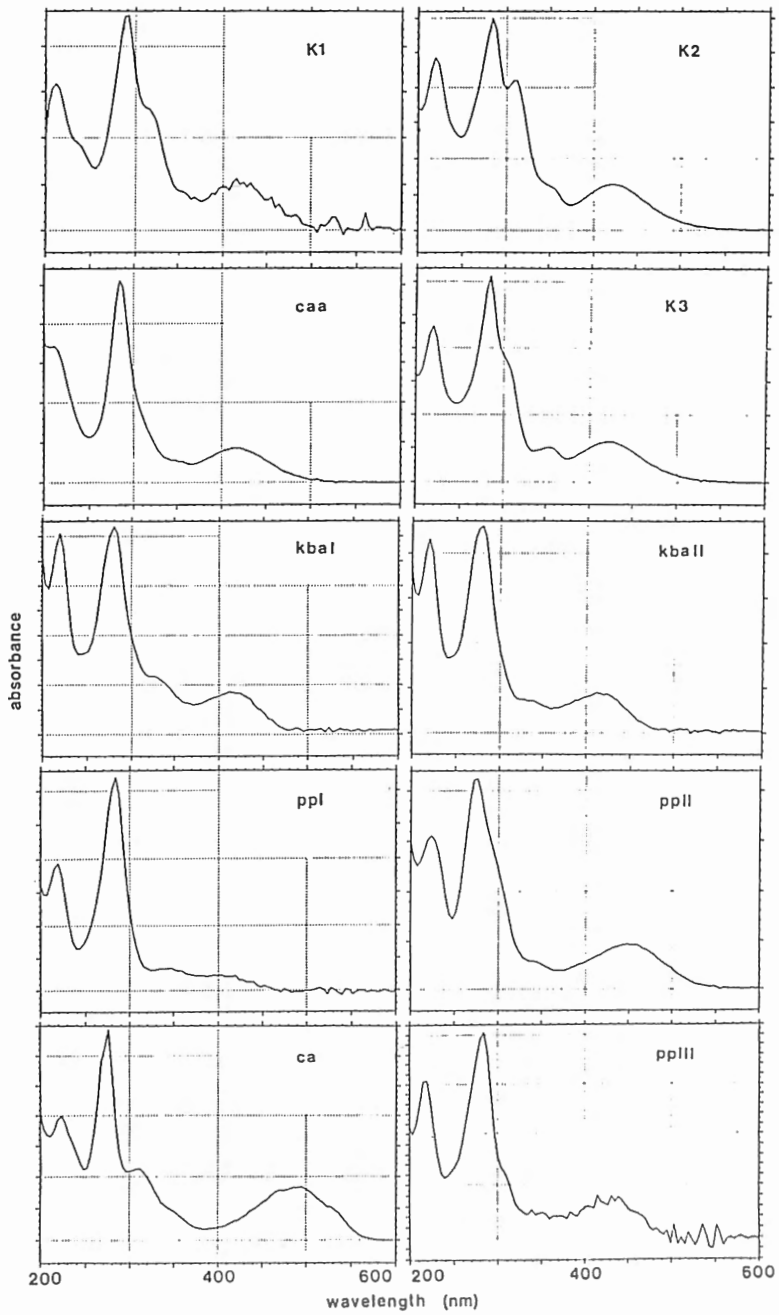

Fig. 6. — Spectral characterisation of individual pigment components from scale insects studied. Abbreviations refer to Table 2.

TABLE 2

Nomenclature, abbreviations, spectral characterisation and relative retention time in described HPLC conditions of the scale insect pigment components. (*) refers to the present paper ; W & V to WOUTERS and VERHECKEN, 1989.

unknown yellow	K1	(*)	1.02
unknown yellow	K2	(*)	1.18
ceroalbolinic acid	caa	THOMSON, 1971	1.33
unknown yellow	K3	(*)	1.59
flavokermesic acid	fk	W & V Fig. 11 e	1.98
kermesic acid	ka	W & V Fig. 11 f	2.03
fk precursor in pp	ppI	W & V Fig. 11 i	0.85
ka precursor in pp	ppII	(*)	0.95
carminic acid	ca	WOUTERS, 1985	1.00
unknown yellow	ppIII	(*)	2.51
fk precursor in kv	kvIa	W & V Fig. 11 g	1.34
ka precursor in kv	kvIb	W & V Fig. 11 h	1.38
unknown red	dcIV	W & V Fig. 11 c	1.52
unknown red	dcVII	W & V Fig. 11 d	1.78
fk precursor in kba	kbaI	(*)	1.32
fk precursor in kba	kbaII	(*)	1.34

CONCLUSION

The high-level analytical capabilities of HPLC allowed us to characterise and quantitate several pigment constituents in scale insects. The combination of the (relative) retention time of a component (Table 2) with its absorption characteristics in the ultraviolet and visible region of electromagnetic radiations (Fig. 6) was used to trace common compositional features in related species, even when the exact chemical nature of a given compound was not known. It should be born in mind however that chemotaxonomic considerations should only be given in close collaboration with entomologists. For example, the striking similarity between *Porphyrophora polonica* and *P. crithmi* is only visible when comparing specimens in the same biological condition. This statement implies that the chromatographer should be aware of possible variations in pigment composition of scale insects as a function of their developmental stage. The detection of these variations, implying the absolute necessity of separation, characterisation and quantification of the pigment components, can at present only be performed by means of HPLC.

ACKNOWLEDGEMENTS

The authors wish to express their sincere gratitude to all persons cited in this paper for donating insect material, and to D. Matile-Ferrero (Laboratoire d'Entomologie, Muséum national d'Histoire naturelle, Paris, France) for identification of scale insects.

REFERENCES

BALACHOWSKY, A. S. (1950) — Remarques biogéographiques sur l'aire de répartition mondiale du G. Kermes (Hom. Coccoidea). *Proc. 8th intern. Congress Entomology*, **156** : 1-5. Stockholm.

BALACHOWSKY A. S. (1950a) — Les Kermes (Hom. Coccoidea) des chênes en Europe et dans le bassin méditerranéen. *Proc. 8th Intern. Congress Entomology*, **155** : 1-16. Stockholm.

BALACHOWSKY, A. S. (1953) — Sur les *Kermes* Boitard (Hom. Coccoidea) des Chênes du Bassin oriental de la Méditerranée. *Revue de Pathologie végétale et d'Entomologie agricole de France*, **32** (3) : 181-189.

BANKS, H. J. (1977) — Pigments of the Coccoidea. Current knowledge and the chemotaxonomic implications. *Bulletin of the Virginian Polytechnic Institute*, **127** : 51-67.

BEN-DOV Y. and I. HARPAZ (1985) — An annotated list of taxa of Coccoidea (Homoptera) described by F. S. Bodenheimer (1897-1959). *Israel J. of Entomology*, **19** : 23-36.

BODENHEIMER, F. S. (1926) — Première note sur les cochenilles de la Syrie. *Bull. Soc. ent. France*, **3-4** : 45-46.

BODENHEIMER, F. S. (1931) — Zur Kenntniss der paläarktischen *Kermes* Arten. *Konowia*, **10** : 241-247.

BROWN, K. S. (1975) — The chemistry of aphids and scale insects. *Chemical Society Reviews*, **4** : 263-288.

CARDON, D. (1990) — Les « vers » du rouge. Insectes tinctoriaux (Homoptera : Coccoidea) utilisés dans l'Ancien Monde au Moyen Age. *Cahiers d'Histoire et de philosophie des sciences*, n. s. **28** : 1-178. Société française d'histoire des sciences et des techniques, Paris.

GADGIL, D. D., A. V. RAMA RAO and K. VENKATARAMAN (1968) — Ceroalbolinic acid. *Tetrahedron Letters*, **18** : 2229-2230.

KOSZTARAB, M. and F. KOZÁR (1988) — Scale insects of Central Europe. *Akadémiai Kiadó* (Budapest), p. 53.

STERNLICHT, M. (1969) — *Kermes bytinskii* n. spec. (Coccoidea, Kermesidae) in Israel and observations on its history. *Israel J. of Entomology*, **4** : 251-270.

STERNLICHT, M. (1980) — The dye of the Coccid. *Israel Land and Nature*, **6** (1) : 17-21.

TAYLOR, G. W. (1988) — The colourants in Israeli Kermes. *Dyes on historical and archeological textiles*, **6** : 23-29. Edinburgh.

THOMSON, R. H. (1971) — Naturally occurring quinones. Edn. 2. Academic Press, London-New York.

VERHECKEN, A. and J. WOUTERS (1990) — The Coccid insect dyes. Historical, geographical and technical data. *Bull. koninkl. Inst. Kunstpatrimonium*, **22** (1988/89) : 207-239, Brussels.

WOUTERS, J. (1985) — High Performance Liquid Chromatography of anthraquinones : analysis of plant and insect extracts and dyed textiles. *Studies in Conservation,* **30** : 119-128.

WOUTERS, J. (1990) — Dyestuff analysis of scale insects by high performance liquid chromatography. *Proceedings of ISSIS-VI* (Cracow) part II : 61-70.

WOUTERS, J. and A. VERHECKEN (1989) — The scale insect dyes (Homoptera : Coccoidea). Species recognition by HPLC and diode-array analysis of the dyestuffs. *Annls. Soc. ent. Fr.* (n. s.) **25** (4) : 393-410.

WOUTERS, J. and A. VERHECKEN (1989a) — The Coccid insect dyes. HPLC and comuterised diode array analysis of dyed yarns. *Studies in Conservation,* **34** : 189-200.

YASCHENKO, R.V. (1900) — Distribution and host-plants of *Porphyrophora polonica* (Homoptera : Coccinea : Margarodidae). *Proceedings of ISSIS VI* (Cracow), pt. 2 : 119-122.