

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC
DETERMINATION OF SOME INSECT GROWTH
REGULATORS--A PRELIMINARY STUDY

File Report No. 28

February 1982

C. Feng and K.M.S. Sundaram

Forest Pest Management Institute
Canadian Forestry Service
Environment Canada
Sault Ste. Marie, Ontario
P6A 5M7

*This report may not be copied
and/or distributed without
the express consent of:*

*Director
Forest Pest Management Institute
Canadian Forestry Service
P.O. Box 490
Sault Ste. Marie, Ontario
P6A 5M7*

TABLE OF CONTENTS

	<i>Page</i>
INTRODUCTION	1
MATERIALS AND METHODS	2
<u>Analytical standards</u>	2
<u>Sample preparation</u>	3
<u>HPLC analysis</u>	4
RESULTS AND DISCUSSION	5
LITERATURE CITED	8
ACKNOWLEDGEMENT	9
Table 1--IGR Structure, etc.	10
Table 2--RT of IGRs	11
HPLC Chromatogram	12

INTRODUCTION

The development of compounds based on the interference with chitin deposition in insects, thus adversely interfering with their normal growth and development, eventually led to the possibility of using these compounds to control forestry pests. These chitin synthesis inhibitors are gaining significance in insect control programs on account of their favourable properties. They are largely selective, spare beneficial insects and display favourable toxicological properties (Hamman and Sirrenberg 1980) so that they are especially suitable for forestry conditions. The benzoylphenyl ureas which interfere with chitin deposition in the endocuticle and thus with the moulting process, thereby differing from classical insecticides by that specific mechanism of action, were first introduced in 1972 (Plimmer 1977) and are referred to as moult inhibiting insect growth regulators (IGRs). Since then a number of benzoylated ureas have been synthesized by various pesticide manufacturers.

The work conducted at this Institute since 1974 demonstrated (Retnakaran 1981) the potential of this class of chemicals in forest pest control programs. To date about 10 compounds (Table 1) have been screened for different types of insects and some of them appear to be candidate materials for possible extensive field evaluation under semi-operational conditions. Prior to such large scale

trials, it is a mandatory requirement that all the released chemicals onto the environment should be monitored for their persistence, distribution and eventual fate. Consequently the development of sensitive analytical methods to isolate, to identify and to quantify the materials at trace levels is a prerequisite for such operations. This report describes some of the preliminary attempts made in identifying some of the benzoyl urea derivatives by using high performance liquid chromatography (HPLC) technique. Although the use of gas-liquid chromatography is an alternative method, as shown earlier (Lawrence and Sundaram 1975, Sundaram and Lawrence 1976) for this group of chemicals, the thermal instability of some of these compounds leading to on column decomposition necessitated cumbersome derivatization methods, consequently to avoid such time consuming and tedious experimental operations, the use of HPLC appeared a convincing possibility for the identification and quantification of this class of compounds.

MATERIALS AND METHODS

Analytical standards

The structural formulae, trade names and the manufacturers of the 10 IGRs (including benzoyl urea) are given in Table 1. Compound No. 3 (PH 60-41) is not a derivative of benzoyl urea. The analytical/technical grade

materials of these compounds were supplied either by Dr. A. Retnakaran at this Institute or by the manufacturers. So far we are able to test only 7 compounds and they are:

Benzoyl urea (No. 1)	(Ref: Table 1)
PH 60-40	(No. 2)
PH 60-43	(No. 4)
PH 60-44	(No. 5)
BAY SIR 8514	(No. 7)
EL-494	(No. 8)
L-7063	(No. 10)

Compounds 3, 6 and 9 are not yet received from the manufacturers. It is hoped that in the near future, when all the analytical standards are in our hands, we will be able to develop and present a multiresidue methodology for all the 10 compounds.

Sample preparation

The solubility of these compounds in non-polar, polar and hydroxy solvents varied considerably. Usually the solubilities in non-polar solvents were low due to their high polarity. Acetonitrile, dimethylformamide and dimethylsulphoxide are some of the suitable polar solvents for these compounds. In our present study, methanol (HPLC grade) was chosen as a solvent for them to optimize the HPLC conditions. Compounds 8 and 10 (Table 1) were very sparingly soluble in methanol. Probably, a more polar solvent should be selected which could act as a single

solvent for all the IGR's planned to investigate. All the compounds were found to be stable in methanol during the entire period (ca. 6 weeks) of this study. Standard solutions of the compounds, except 8 and 10) due to their poor solubility), contained 1000 ppm (1 mg/ml) levels which were subsequently diluted as required.

HPLC analysis

For this study, a Hewlett Packard Model 1084B high performance liquid chromatography equipped with a variable wavelength UV detector was used. The operating parameters were as follows:

Column: Whatman Partisil PXS 10/25 (4.6 mm ID) ODS-2

Column pressure: 1100 psi

Mobile solvent system: 80% methanol in water at 1 ml/min

Oven temperature: 23°C

UV detector wavelength: 254 nm

Sample size: 5 µl of standard solution corresponding to
5 ppm of the IGRs

Under these conditions the solutions of each IGR containing the optimum concentration (5 ppm) was injected 3 times separately and the retention time (RT) for each compound was established. All the IGRs studied gave well defined sharp single peaks. The stability of the instrument was excellent throughout the study.

RESULTS AND DISCUSSION

The RTs of the IGRs studied are given in Table 2 and the actual chromatogram obtained is given in Fig. 1. It is apparent from Fig. 1 that all the 7 compounds studied gave well defined sharp peaks indicating that the HPLC is a viable tool to be exploited for the development of a suitable residue methodology for these compounds present in forestry substrates.

Different solvent systems (mobile phase selectivity) coupled with gradient methods are planned to examine not only to separate the RTs of PH 6040 and PH 6044 and PH 6043 and BAY SIR 8514 which gave overlapping peaks but also to improve the sensitivity of quantification in reverse phase HPLC.

From the eluting pattern obtained in the HPLC column (Whatman Partisil PXS 10/25 ODS-2) using 80:20 CH₃OH/water as solvent system, it is evident that benzoyl urea, because of its low RT, (3.35 min) is, (relatively speaking) the most soluble in the solvent system and the EL 494 (RT 18.58 min) is the least soluble in the series. An examination of the structural patterns of these compounds (Table 1) confirm this empirical observation made in this study. Assigning the numerical value of 1 to benzoyl urea to represent its solubility (solubility factor SF = 1), the SF values of other compounds are:

<u>Compound</u>	<u>SF (RT of benzoyl urea/RT of x)</u>
Benzoyl urea	1.00 (RT 3.35 min)
PH 6040	0.39 (RT 8.52 min)
PH 6044	0.39 (RT 8.62 min)
PH 6043	0.34 (RT 9.90 min)
BAY SIR 8514	0.34 (RT 9.92 min)
L 7063	0.20 (RT 17.17 min)
EL 494	0.18 (RT 18.58 min)

The SF values obtained in this study clearly demonstrate the close structural similarities of PH 6040 and PH 6044 (Ar-Cl and Ar-CF₃), PH 6043 and BAY SIR 8514 (Ar-CF₃ and Ar-O-CF₃) and the expected low solubility of EL 494 over L 7063 due to its increased molecular mass because of the presence of an additional Cl on the aryl ring of the benzoyl moiety. Generally the RT increases with diminishing solubility of IGRs in CH₃OH-H₂O system.

To a first approximation from this preliminary study we can say that: 1) the eluting pattern of the IGRs in the chosen solvent system is related to their solubilities in it, i.e., benzoyl urea is more soluble than EL 494 and, 2) the molecular structure of the sample determines the elution order; greater the complexity (steric effect, type and number of functional groups, etc.) higher the RT. Further work is planned to bring out not only the structural aspects of this series of compounds in relation to their elution patterns and retentions but also their hydrophobic

and associated steric properties of these molecules and adsorption/desorption phenomena on non-polar stationary phases. Such indepth studies will not only lead to a successful methodology development but also would yield valuable information on the structural and stereochemical properties of these molecules.

Our preliminary studies show that IGRs as a group respond extremely well to HPLC technique. Each compound could be distinguished from others and with additional research, a viable residual methodology could be developed for them which could be used in environmental monitoring and accountability studies should these compounds be tried in operational and/or in large scale experimental spray programs.

LITERATURE CITED

- Hammann, I. and W. Sirrenberg. 1980. Laboratory Evaluation of BAY SIR 8514, a New Chitin Synthesis Inhibitor of the Benzoylated Urea Class. Pflanzenschutz Nachrichten Bayer, 33(51):1-28.
- Lawrence, J.F. and K.M.S. Sundaram. 1975. Gas Chromatographic Analysis of TH 6040 Insecticide After Chemical Derivatization. Jour. Assoc. Official Anal. Chem. 59(4):938-41.
- Plimmer, J.R. 1977. Pesticide Chemistry in the 20th Century. ACS Symp. Series 37, pp. 237-270.
- Retnakaran, A. 1981. Toxicology and Efficacy of Insect Growth Regulators Aerially Applied Against the Spruce Budworm. Env. Canada For. Serv. For. Pest Mngmt Inst. Rept FPM-X-45, 63 pp.
- Sundaram, K.M.S. and J.F. Lawrence. 1976. GLC Analysis of TH 6040 Insecticide After Chemical Derivatization. Can. Chem. Congr. Chem. Inst. Canada Abstr. Papers 59:9.

ACKNOWLEDGEMENT

The authors are grateful to Dr. A. Retnakaran, and his staff, Messrs Larry Smith and Bill Tomkins for providing the necessary materials, timely help and support in this work.

TABLE 1

MOULT INHIBITING INSECT GROWTH REGULATORS

INVESTIGATED UP TO 1980

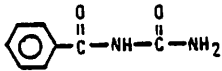
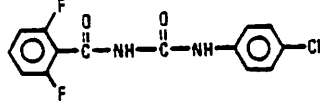
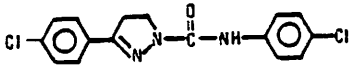
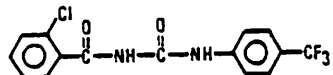
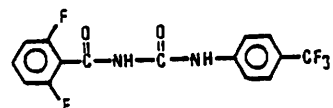
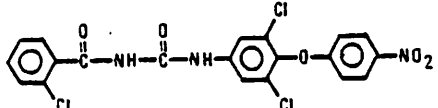
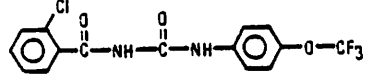
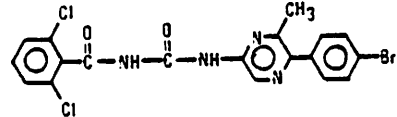
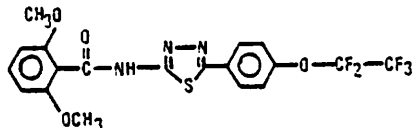
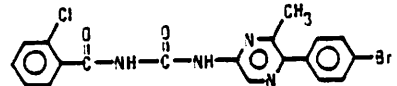
NUMBER	CHEMICAL STRUCTURE	COMPOUND	MANUFACTURER
1		BENZOYL UREA	-
2		PH 60-40	PHILIPS-DUPHAR
3		PH 60-41	"
4		PH 60-43	"
5		PH 60-44	"
6		BAY SIR 6874	CHEMAGRO LIMITED
7		BAY SIR 8514	"
8		EL-494	ELI LILLY & CO.
9		L-1215	"
10		L-7063	"

Table 2. RT of IGRs Studied

Compound	RT (min)
Benzoyl urea	3.35
PH 6040	8.52
PH 6044	8.62
PH 6043	9.90
BAY SIR 8514	9.92
L 7063	17.17
EL 494	18.58

Fig 1. Quantification of IGR on HPLC

