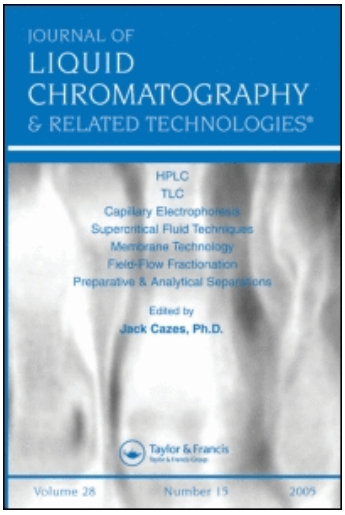

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Multiresidue Analysis of Some Insect Growth Regulators by Reversed-Phase High-Performance Liquid Chromatography

C. C. Feng[a]; K. M. S. Sundaram[a]
[a] Environment Canada Canadian Forestry Service, Forest Pest Management Institute,

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MULTIRESIDUE ANALYSIS OF SOME INSECT GROWTH REGULATORS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY.

C.C. Feng and K.M.S. Sundaram

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

ABSTRACT

A general procedure has been developed for the analysis of 8 different insect growth regulators (IGRs) by using reversed-phase high-performance liquid chromatography with gradient solvent systems. The method has been used to identify and separate 8 insect growth regulators from a mixture of the standards. The method has been evaluated with different column conditions and under different solvent systems. Best resolution was obtained by using a double column and methanol/water gradient system.

INTRODUCTION

Chitin synthesis inhibitors are gaining significance in insect control programs because of their favourable toxicological properties (1,7). Consequently, they are being evaluated extensively for controlling forestry pests. The benzoylphenyl ureas differ in their mechanism of action to conventional insecticides. They interfere with chitin deposition in the endocuticle, thus

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affecting the moulting process. These chemicals were first introduced in 1972 (3) 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.

Work conducted at this Institute since 1974 has demonstrated the potential of this class of chemicals in forest pest control programs (4). To date, about 8 compounds (Table 1) have been screened for different types of forestry insects and some of them appear to be candidate materials for controlling spruce budworm, *Choristoneura fumiferana* (Clem.). The persistence, distribution and eventual fate of all chemicals released into the environment must be monitored. Consequently, the development of sensitive analytical methods to isolate, identify and quantify the materials at trace levels is a prerequisite for any such operation.

The gas chromatographic method has been the major way of analyzing trace levels of pesticide residues in biological and environmental samples since the mid-1950's. Most residue analysis procedures officially recognized by various governmental organizations employ gas chromatography. However, many of the newer types of pesticides, carbamates and insect growth regulators for example, are difficult to quantitate by gas chromatography. This is due to their nonvolatility and thermal instability. Although derivatization may overcome most of these problems (2,7), it is a rather time-consuming procedure. Also, this additional step usually introduces more experimental errors. During the past two decades the application of high-performance liquid chromatography (HPLC) to residue analysis has been expanded greatly and has become very popular.

Schaefer and Dupras (5,6) used the HPLC technique successfully to isolate BAY SIR 8514 (2-chloro-N-[[[4-(trifluoromethoxy) phenyl]amino]carbonyl]benzamide) and PH 60-40 or Dimilin® (2,6-difluoro-N-[[[4-chlorophenyl]amino]carbonyl]benzamide) from water and vegetation. This paper describes a multiresidue, reversed-phase HPLC method developed to identify and separate eight benzoyl urea derivatives from a mixture (Table 1). Due to the thermal

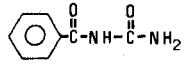
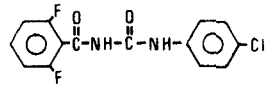
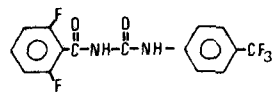
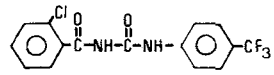
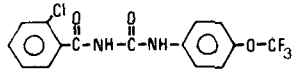
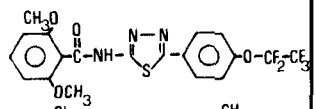
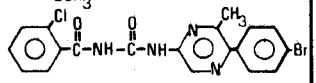
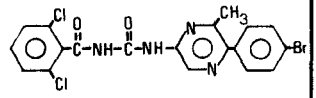
instability of some of these compounds, the use of HPLC appeared to be a convincing possibility for the identification and quantification of this class of compounds.

MATERIALS

The structural formulae, trade names and the manufacturers of the 8 IGRs are given in Table 1. The analytical grade materials

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 60-44	"
4		PH 60-43	"
5		BAY SIR 8514	CHEMAGRO LTD.
6		L-1215	ELI LILLY & CO.
7		L-7063	"
8		EL-494	"

of these compounds used in the present study were supplied by the respective manufacturers.

All solvents (HPLC grade from J.T. Baker Chemical Co.) were filtered through appropriate Millipore filters and degassed prior to use. All compounds were stable in methanol and acetonitrile during the entire period (*ca.* 6 weeks) of this study. Standard stock solutions of the compounds were prepared in methanol and acetonitrile and subsequently diluted as required. All standards prepared were filtered through Millipore filters prior to injection into the HPLC system.

METHODS

A Hewlett-Packard model 1084B high-performance liquid chromatograph equipped with a variable wavelength detector (190-600 nm), microprocessor and electronic integrator was used for this study. The instrument also employed an automatic degassing system, dual solvent system and dual pumpheads with common drive which gave stable and reproducible flows. A Hewlett-Packard LC terminal (79850B) provided the chromatogram, area, area %, retention time (R.T.), etc., for each peak. The operating parameters were as follows:

Columns: (a) Hewlett-Packard RP-8, 10 µm, 20 cm x 4.6 mm ID.
(b) Hewlett-Packard RP-8, 7 µm, 10 cm x 4.6 mm ID.
(c) 2 of (a) connected together as a double column.

Column Pressure: 16-78 bars. (1 bar = 14.5 psig.)

Mobile Systems (V/V): (a) CH_3OH/H_2O
(b) CH_3CN/H_2O

Flow Rate: 1 ml/min and 1.5 ml/min.

Oven Temperature: ambient (24 ± 1°C)

Variable Wavelength: Sample (S):Reference (R) = 254:430 nm

Injection System: Rheodyne model 7120 syringe loading injector with 20 µl loop size.

Sample Size: 20 µl of 100 µg/ml standard stock solution.

Chart Speed: 0.1 cm/min.

Attenuation: 2^6.

Slope Sensitivity: 0.2.

An isocratic mobile system (Table 2) has been developed and used to obtain the basic chromatograms of 8 IGRs (Figs. 1 and 3) by the two solvent systems (CH_3OH/H_2O and CH_3CN/H_2O) chosen. Use of suitable gradient elution systems (Table 3) improved the resolution and separation of these compounds. The solutions of each IGR and their mixtures were injected several times to obtain reproducible results. The chromatograms obtained were well defined, having sharp peaks and a deviation in retention time (R.T.) for each injection of <1%. Under these experimental conditions, using the mixed standard, the minimum detection limit (MDL) for each IGR was found to be 10 ng. The stability of the instrument throughout the entire study was excellent.

TABLE 2

R.T. of IGRs Studied by Using a RP-8, 10 μm Column With Different Isocratic Solvent Systems. Flow Rate 1 ml/min.

No.	Compound	R.T. (min) $CH_3OH:H_2O$ = 65:35	R.T. (min) $CH_3OH:H_2O$ = 80:20	R.T. (min) $CH_3CN:H_2O$ = 65:35	R.T. (min) $CH_3CN:H_2O$ = 50:50
1	Benzoyl urea	3.37	2.91	2.85	3.21
2	PH 60-40	9.40	3.96	4.98	11.36
3	PH 60-44	11.43	4.03	5.49	14.56
4	PH 60-43	12.44	4.23	5.85	16.20
5	BAY SIR 8514	13.22	4.30	5.98	16.88
6	L-1215	16.61	4.32	6.21	19.90
7	L-7063	19.35	5.05	7.10	21.57
8	EL-494	25.39	5.47	8.27	29.19

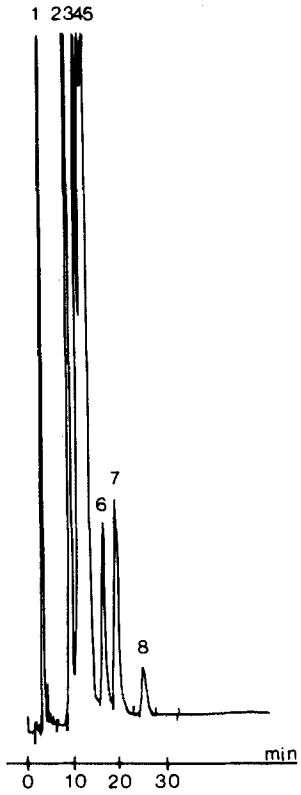


Figure 1. Separation of 8 IGR compounds using HPLC.
Column: RP-8, 10 μm.
Flow rate: 1 ml/min.
Solvent system: $CH_3OH:H_2O$ = 65:35.
Numbers on the chromatograms correspond to the IGRs given in Table 4.

RESULTS AND DISCUSSION

The average R.T.s of the IGRs studied are given in Tables 2, 4, 5, 6 and 7. The actual chromatograms obtained are given in Figs. 1-4. It is apparent from Table 2 and Fig. 2 that all the 8 IGRs 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.

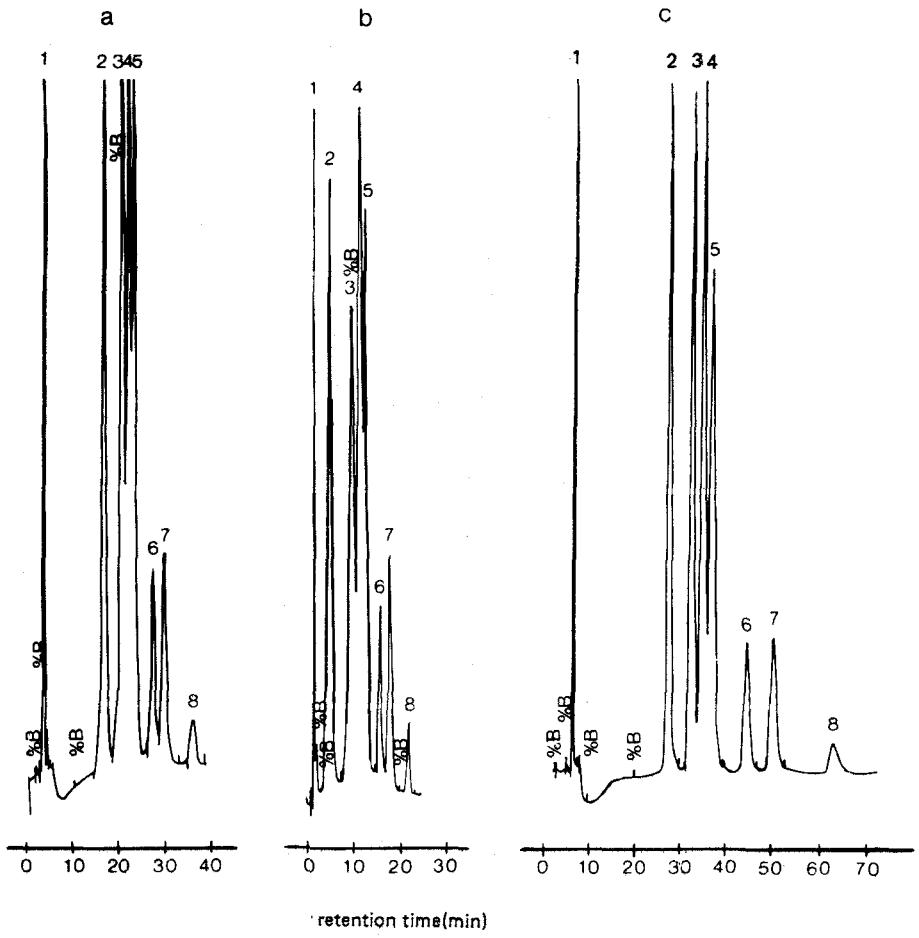


Figure 2. Separation of 8 IGRs by using 3 different HPLC columns.
(a) RP-8, 10 µm column.
(b) RP-8, 7 µm column.
(c) 2 of (a) connected together as a double column.
Flow rate: 1 ml/min.
Solvent systems: see Table 3A.
Numbers on the chromatograms correspond to the IGRs given in Table 5.

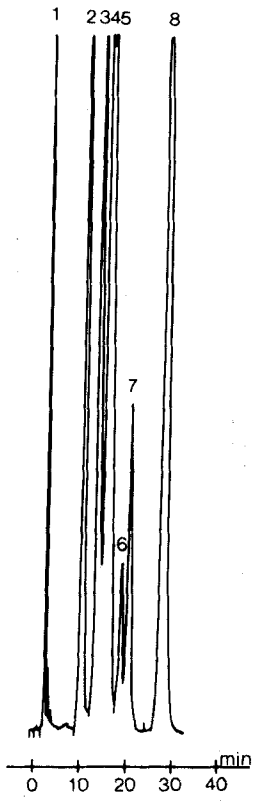


Figure 3. Separation of 8 IGR compounds using HPLC.
Column: RP-8, 10 μm.
Flow rate: 1 ml/min.
Solvent system: $CH_3CN:H_2O$ = 50:50.
Numbers on the chromatograms correspond to the IGRs given in Table 6.

The solubilities of these compounds in solvents such as acetonitrile, methanol and water varied considerably. Because of these differences, these solvents were found to be suitable in the present study to optimize the various HPLC conditions used.

Using 65:35 CH_3OH/H_2O as the solvent system, the eluting pattern obtained in the HPLC column (RP-8, 10 μm) showed that benzoyl urea, because of its low R.T. (3.37 min) is comparatively

TABLE 3

Solvent Systems for Separation of 8 IGRs.

Solvent System	Flow rate (ml/min)	Elution (min)	Time (min)	Methanol (%)	Water (%)
A	1	25-70	2	65	35
			3	50	50
			10	60	40
			20	65	35
Solvent System	**Flow rate (ml/min)**	**Elution (min)**	**Time (min)**	**Acetonitrile (%)**	**Water (%)**
B	1	60-80	3	45	55
			10	30	70
			20	25	75
			30	30	70
			40	45	55
			80	50	50
Solvent System	**Flow rate (ml/min)**	**Elution (min)**	**Time (min)**	**Acetonitrile (%)**	**Water (%)**
C	1.5	100	3	45	55
			10	30	70
			20	25	75
			30	30	70
			40	45	55
			80	45	55
			85	50	50
			90	55	45

the most soluble compound in this solvent system and EL-494 (R.T. 25.39 min) is the least soluble. This is also apparent from the other solvent systems (Table 2) used in the study. An examination of the structural patterns (polarity, molecular size, complexity, etc.) of the compounds (Table 1) qualitatively confirm this observation. Assigning a numerical value of 1 to benzoyl urea to

TABLE 4

Retention Times of 8 IGRs for Fig. 1.

No.	Compound	R.T. (min)
1	Benzoyl urea	3.37
2	PH 60-40	9.40
3	PH 60-44	11.43
4	PH 60-43	12.44
5	BAY SIR 8514	13.22
6	L-1215	16.61
7	L-7063	19.35
8	EL-494	25.39

TABLE 5

Retention Times of 8 IGRs for Fig. 2.

No.	Compound	Retention Time (min)		
		a	b	c
1	Benzoyl urea	3.37	1.73	6.67
2	PH 60-40	16.39	5.05	27.60
3	PH 60-44	20.23	9.95	32.48
4	PH 60-43	21.61	11.64	34.86
5	BAY SIR 8514	22.69	12.76	36.76
6	L-1215	27.02	16.08	44.69
7	L-7063	29.41	18.20	50.51
8	EL-494	36.00	22.24	63.44

TABLE 6

Retention Times of 8 IGRs for Fig. 3.

No.	Compound	R.T. (min)
1	Benzoyl urea	3.21
2	PH 60-40	11.36
3	PH 60-44	14.56
4	PH 60-43	16.20
5	BAY SIR 8514	16.88
6	L-1215	19.90
7	L-7063	21.57
8	EL-494	29.19

TABLE 7

Retention Times of 8 IGRs for Fig. 4.

No.	Compound	Retention Time (min) a	b	c
1	Benzoyl urea	3.43	1.77	6.12
2	PH 60-40	43.64	11.55	47.80
3	PH 60-44	50.71	40.29	57.66
4	PH 60-43	53.41	42.52	61.93
5	BAY SIR 8514	54.78	43.56	64.21
6	L-1215	61.65	48.58	76.50
7	L-7063	61.65	48.58	76.50
8	EL-494	71.81	56.42	92.11

represent its solubility (solubility factor SF = 1), the SF values of other compounds in 65:35 CH_3OH/H_2O solvent system are:

Compound	SF (R.T. of Benzoyl urea/R.T. of x)
Benzoyl urea	1.00 (R.T. 3.37 min)
PH 60-40	0.36 (R.T. 9.40 min)
PH 60-44	0.29 (R.T. 11.43 min)
PH 60-43	0.27 (R.T. 12.44 min)
BAY SIR 8514	0.25 (R.T. 13.22 min)
L-1215	0.20 (R.T. 16.61 min)
L-7063	0.17 (R.T. 19.35 min)
EL-494	0.13 (R.T. 25.39 min)

The SF values obtained from this study strongly demonstrated the close structural similarities of PH 60-40 versus PH 60-44 (Ar-Cl versus Ar-CF_3) and PH 60-43 versus BAY SIR 8514 (Ar-CF_3 versus Ar-O-CF_3). Also the low solubility of EL-494 compared to L-7063 is due to its increased molecular mass, because of the presence of an additional Cl on the aryl ring of the benzoyl

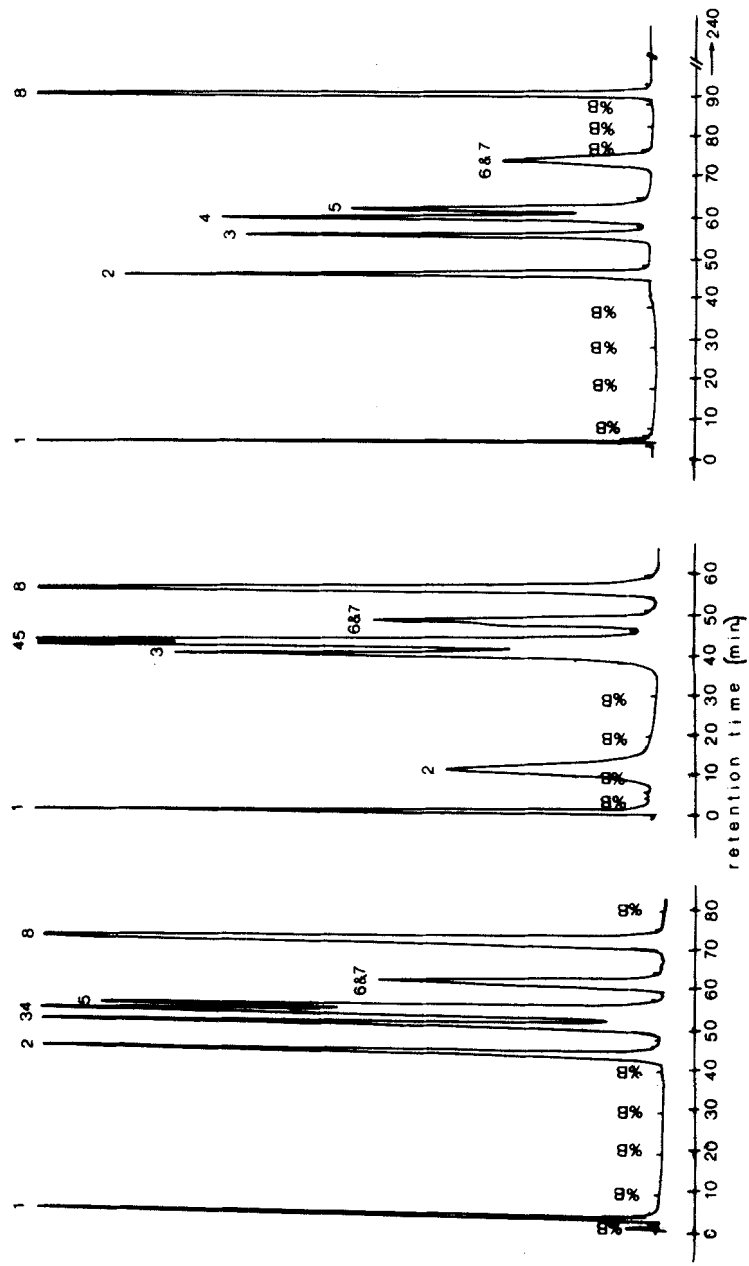


Figure 4. Separation of 8 IGRs by using 3 different HPLC columns. (a) RP-8, 10 µm column, solvent system B (Table 3). (b) RP-8, 7 µm column, solvent system B (Table 3). (c) 2 of (a) connected together as a double column, solvent system C (Table 3). Numbers on the chromatograms correspond to the IGRs given in Table 7.

moiety. Generally, the R.T. increases with diminishing solubility of the IGRs in both solvent systems used in this study.

The resolution of compounds in a chromatogram is determined by the type of column used and by its efficiency. Three different types of columns [(a) Hewlett-Packard RP-8, 10 μm, 20 cm x 4.6 mm I.D., (b) Hewlett-Packard RP-8, 7 μm, 10 cm x 4.6 mm I.D., (c) 2 of (a) connected together as a double column] along with two different solvent systems (CH_3OH/H_2O and CH_3CN/H_2O, both with or without gradient elution systems) (Tables 2 and 3), were tried to resolve all the eight IGRs satisfactorily (Figs. 1-4) and also to improve the separations among the 4 compounds namely, PH 60-40, PH 60-44, PH 60-43 and BAY SIR 8514.

It is evident (Fig. 1, Table 4) that by using the RP-8, 10 μm column and 65:35 CH_3OH/H_2O isocratic solvent system, the separation of these four IGRs (Peaks 2-5) was poor. Similar results were also obtained (Fig. 3, Table 6) by using CH_3CN/H_2O (50:50) gradient system. However, by choosing the gradient solvent system (Figs. 2a, 4a, Tables 5a, 7a), the same column showed a slightly better separation for all the IGRs.

Use of a 7 μm RP-8 column instead of the 10 μm RP-8, together with the gradient solvent system, gave a relatively good separation with low R.T.s (Figs. 2b, 4b, Tables 5b, 7b) for all the 8 IGRs studied. The minor drawback in this column is the poor resolution of the two structurally similar compounds *viz.* PH 60-43 and BAY SIR 8514.

The chromatogram in Fig. 2c was obtained by connecting in series two of the RP-8, 10 μm columns and using the CH_3OH/H_2O gradient solvent system (Table 3A) to elute the samples. Similar double column arrangement and the use of CH_3CN/H_2O gradient solvent system (Table 3C) yielded the chromatogram recorded in Fig. 4c. In this set up, the compounds L-1215 and L-7063 (Table 1) gave a single peak (Figs. 4a, 4b and 4c) and the elution was also longer compared to the CH_3OH/H_2O system. While comparing the two gradient solvent systems used in this study, it is evident that a double column (RP-8, 10 μm) with CH_3OH/H_2O gave the best resolution for all the 8 IGRs.

This study indicates that (1) the eluting pattern of the IGRs in the chosen solvent system is relatable to their solubilities in the system, i.e., benzoyl urea is more soluble than EL-494; (2) the molecular structure of the sample determines the elution order; the greater the complexity (steric effect, type and number of functional groups, etc.) the higher the R.T.; (3) a methanol/water gradient solvent system with a double column gives the best resolution; (4) the lowest amount of IGR we could detect in this study is 10 ng and (5) IGRs as a group respond extremely well to the HPLC technique reported herein and could be used as an analytical tool in the identification and quantification of these compounds from environmental samples.

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