Assignments of ^1H and ^{13}C NMR signals for **1**, **3**, and **4***

	1 ^1H NMR (δ)	^{13}C NMR (δ)	**3** ^1H NMR (δ)	**4** ^1H NMR (δ)	^{13}C NMR (δ)
C-1		152.8s			153.9s
C-2	6.60br s	116.4d	6.64br s	6.59br s	116.2d
C-3		131.6s			131.8s
C-4	6.70br d(7)**	121.8d	6.73br d(7)	6.70br d(7)	120.9d
C-5	7.05br d(7)	126.9d	7.04br d(7)	7.04d(7)	126.9d
C-6		136.4s			135.8s
C-7	3.00m	31.5d	3.18m	3.14m	31.1d
C-8	1.90m	26.2t	5.73dd(14, 6)	1.45m	22.1t
C-9	1.90m	37.4t	6.38brdd(14, 9)	1.45m	37.6t
C-10	5.15dd(7, 7)	124.8d	5.87m	1.45m	43.4t
C-11		130.4s			71.6s
C-12	1.65s	25.7q	1.74s	1.18s	29.0q
C-13	1.50s	17.7q	1.74s	1.18s	28.4q
C-14	1.21d(7)	21.2q	1.40d(7)	1.23d(7)	21.0q
C-15	2.25s	20.8q	2.26s	2.25s	20.7q

* Measured in $CDCl_3$; ** coupling constant in Hz.

3. *E* geometry for the $\Delta^{8,9}$ double bond was deduced from a coupling constant of 14 Hz between H-8 and H-9. Due to the instability as well as scarcity of **3**, the stereochemistry at C-7 has not been determined.

A more polar fraction obtained by silica gel column chromatography was purified by reversed-phase HPLC to yield **4** (290 mg). Compound **4**, $[\alpha]_D^{23}+1.3°$ (*c* 5.92, $CHCl_3$), has a UV spectrum [λ_{max}(MeOH) 278 nm (ε 2000)], which is superimposable on that of **1**, while the IR spectrum showed more intense OH absorption than did **1**. The molecular formula of $C_{15}H_{24}O_2$ was obtained by EIMS(*m/z* 236) as well as by ^1H and ^{13}C NMR (table). The ^1H NMR spectrum revealed no signals for olefinic protons or two olefinic methyls, which were seen in **1**. Instead, there were a methyl singlet (6H) at δ 1.18 and a 6H broad multiplet centered at δ1.45. The ^{13}C NMR spectrum also secured these structural features; an oxygen-bearing quaternary carbon at δ71.6, three methylene carbons at δ22.1, 37.6, and 43.4, and two methyl signals at δ28.4 and 29.0. These spectral features led us to assign an 11-hydroxy-10,11-dihydrocurcuphenol structure to **4**. This structure, including the configuration at C-7, was confirmed by chemical conversion of **4** with $SOCl_2$/py (rt, overnight)[7] to **1**, which was identical with natural **1** in every respect. Thus **4** possesses 7*S* configuration.

(+)-Curcuphenol and dehydrocurcuphenol inhibit the activity of gastric H,K-ATPase with an IC_{50} of 8.3×10^{-6} M and 2.3×10^{-5} M, respectively, while **4** is inactive. The two active compounds also inhibited gastric acid secretion in rats. (+)-Curcuphenol and dehydrocurcuphenol are the first marine natural products that inhibit H,K-ATPase activity. It is interesting that the sponge sesquiterpenes had stereochemistry (*S*) opposite to the gorgonian analog.

1 Acknowledgment. Thanks are due to Professor Paul J. Scheuer, University of Hawaii, for reading this manuscript. We are indebted to Professor P.R. Bergquist, University of Auckland, for identification of the sponge. We thank Mr M. Shimizu of the Central Research Laboratories of Yamanouchi Pharmaceutical Co. Ltd. for HREIMS measurements. We are also indebted to Dr H. Shikama and Ms. A. Ohta of the same laboratories for bioassays.

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0014-4754/87/11/121234-02$1.50 + 0.20/0

Insecticidal organophosphates: Nature made them first

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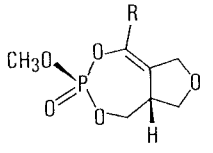
Summary. Out of the three most important classes of synthetic insecticides only the carbamates and pyrethroids were known to have ancestors in nature. Now two organophosphates (which are quite good insecticides and very potent acetylcholinesterase inhibitors, e.g. comparable to carbofuran) have been isolated from *Streptomyces antibioticus* strain DSM 1951.
Key words. Organophosphates; natural insecticides; acetylcholinesterase inhibitors; *Streptomyces*.

Today, organophosphates, carbamates and pyrethroids are the three most important classes of synthetic insecticides. The first two interfere with nervous transmission by inhibiting the acetylcholinesterases (AChE), the last by blocking the sodium channels. The first insecticidal organophosphates were invented by Schrader and Kükenthal in 1937[1]. On the other hand, the first insecticidal carbamates were synthesized on purely chemical grounds[2], and as analogues of physostigmine[3], a toxic alkaloid from the seeds of the vine *Physostigma venenosum*[4]. Earlier experiments[5] had indicated that physostigmine interfered with the AChE in the same way as the organophosphates. The third class, the pyrethroids, is derived form the natural pyrethrins, a mixture of insecticidal esters obtained from the flowers of certain *Chrysanthemum* species, especially *C. cinerariaefolium*. The first written accounts date back to the 17th century and commercial production of an insecticidal powder ground from the heads of these flowers started around 1840[6].

So two out of the three classes, the carbamates and the pyrethroids, have their relatives in nature and could therefore be called natural insecticides. We now report that the organophosphates were also being produced by nature long before men knew about their effects.

Materials and methods. The natural isomers of CGA 134736 and CGA 134735 were isolated from cultures of the soil microorganism *Streptomyces antibioticus* strain DSM 1951. The structural formulas of the two compounds are depicted in the figure. Fermentation conditions, isolation and characterization of the two highly crystalline substances will be reported elsewhere. The absolute configuration of CGA 134736, the major component, was determined by X-ray crystallographic analysis[7]. A sample of a racemic mixture of CGA 134736, obtained by chemical synthesis, was kindly provided by Prof. A. Zeeck, Laboratory for Organic Synthesis, University of Göttingen, FRG. Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) and monocrotophos (dimethyl (*E*)-1-methyl-2-(methylcarbamoyl) vinyl phosphate) were the analytical standards (Ciba-Geigy).



CGA 134'736 R = $-CH_3$

CGA 134'735 R = $-nC_3H_7$

Chemical structure of the acetycholinesterase inhibitors isolated from cultures of *Streptomyces antibioticus* strain DSM 1951.

The enzymes were either bought (AChE of bovine erythrocytes from Sigma) or freshly prepared (homogenates of heads from the multi-resistant strain R-300 of the housefly *Musca domestica*). An automated procedure[8] was used for the AChE inhibition experiments. The enzymes were preincubated with the inhibitors for 65 s and the remaining activity was measured according to Ellman et al.[9] with acetylthiocholine (10^{-3} M) as the substrate (pH 8.0, 37 °C).

The insecticidal activities were determined by spraying infested shoots (*Aphis craccivora,* the cowpea aphid) or by placing larvae on treated diet (*Cydia pomonella,* the codling moth) or on treated leaves (an organophosphate-resistant strain of *Spodoptera littoralis,* the Egyptian cotton leafworm, or a sensitive strain of *Heliothis virescens,* the tobacco budworm).

Results. A comparison of the potencies of the two natural organophosphates as AChE inhibitors (table 1) shows that they are as active as the carbamate carbofuran and by four orders of magnitude better than the phosphate monocrotophos. The synthetic racemate of CGA 134736 showed somewhat higher I_{50}-values.

Table 2 compares the insecticidal activities of the two natural compounds. These organophosphates show the same good activity against *A. craccivora* and *C. pomonella* as carbofuran or monocrotophos, the methyl analogue CGA 134736 being slightly weaker. Both compounds are only weakly active against third instar larvae of the cotton feeding lepidoptera *H. virescens* and *S. littoralis.* The new compounds were at higher concentrations still effective against the organophosphate resistant strain which is very resistant to monocrotophos.

Discussion. One way of grouping insecticides is by looking at their origin. In the early days, there were the natural insecticides like nicotine, the pyrethrins and rotenone, and the man-made inorganic insecticides. The first synthetic organic insecticides, the chlorinated hydrocarbons and the organophosphates, were found by serendipity. The natural origin and the mode of action of the carbamates was already known at the time they were optimized for their use as insecticides. Today's pyrethroids developed out of the pyrethrins by steady modifications of the parent molecules. The inorganic insecticides and most of the chlorinated hydrocarbons are no longer used to control insects. Thus, the organophosphates were the last major group of insecticides with no known relative in nature. This gap has now been filled as the data in tables 1 and 2 show. Remarkably enough, the first natural organophosphates isolated compare very favorably with the best man-made compounds in their ability to inhibit AChEs and to control insects. The I_{50}-values are surprisingly low for organophosphates, e.g. four orders of magnitude lower than the values of monocrotophos. *Streptomyces* also synthesizes the more active isomer, since the synthetic racemate of CGA 134736 was less active in inhibiting AChE.

In summary, thanks to *Streptomyces,* three of the major classes of insecticides that are in use today are now linked to naturally occurring compounds.

Table 1. Inhibition potencies with two acetylcholinesterases as compared with an organophosphate and a carbamate insecticide

Inhibitors	AChE I_{50}-values (M) Houseflies strain R-300	Bovine erythrocytes
CGA 134736 opt. isomer	2.0×10^{-7}	0.9×10^{-7}
racemate	2.4×10^{-7}	1.4×10^{-7}
CGA 134735 opt. isomer	1.0×10^{-7}	5.7×10^{-7}
Carbofuran	1.1×10^{-7}	0.7×10^{-7}
Monocrotophos	1.0×10^{-3}	1.2×10^{-3}

Table 2. Toxicological activities against four insect species as compared with an organophosphate and a carbamate insecticide

Insecticide	*Aphis craccivora*	*Cydia pomonella* L_1	*Heliothis virescens* L_3	*Spodoptera littoralis* L_3, OP-res.
CGA 134736 opt. isomer	**	**	–	*
CGA 134735 opt. isomer	***	***	–	*
Carbofuran	***	***	–	–
Monocrotophos	***	***	**	–

*** good activity at 10 ppm; ** good activity at 50 ppm; * good activity at 200 ppm; – not or weakly active at 200 ppm.

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0014-4754/87/11/121235-03$1.50 + 0.20/0

5,6-Dichloroindole-3-acetic acid as a potent auxin: its synthesis and biological activity

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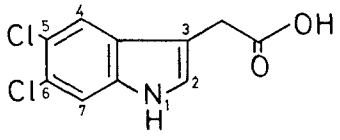
Summary. 5,6-Dichloroindole-3-acetic acid (**1**), a new auxin, has been synthesized by Fischer's indolization. It showed the strongest auxin activity among all the known natural and synthetic auxins in three bioassays (elongation of *Avena* coleoptiles, hypocotyl growth inhibition of Chinese cabbage, and hypocotyl swelling of mung bean seedlings). It induced many lateral roots in mung bean seedlings, and resisted peroxidase-catalyzed decomposition.
Key words. 5,6-Dichloroindole-3-acetic acid; indole-auxin; *Avena* elongation; hypocotyl swelling; lateral root formation; peroxidase oxidation.

Our idea for the synthesis of chloro-derivatives of indole-3-acetic acid originated from our previous isolation of 4-chloroindole-3-acetic acid(4-Cl-IAA) and its methyl ester from immature seeds of *Pisum sativum*[1,2]. The isolated 4-Cl-IAA showed strong auxin activity as compared with that of IAA, e.g., 10-fold for the elongation of *Avena* coleoptiles[3,9], 100-fold for the root growth inhibition of Chinese cabbage[3], and more than 100-fold for the hypocotyl swelling of mung bean[3]. The high biological activity of 4-Cl-IAA was partly ascribed to its relative lack of susceptibility to peroxidase oxidation[3]. Fox and Bullock synthesized monochloro-derivatives of IAA including 4-Cl-IAA[4], and Porter and Thimann found that 4- and 6-Cl-IAAs were more active than 5-, 7- and 2-Cl-IAAs[5]. Biological activity of monochloro-IAAs were also investigated by Hoffmann et al.[6], Sell et al.[7], Engvild[8], Böttger et al.[9] and Katekar and Geissler[9,10]. Engvild synthesized dichloro-IAAs, e.g., 4,6-, 4,7-, 5,7- and 6,7-Cl_2-IAAs[12]. Auxin activity of his synthesized dichloro-IAAs was, however, shown to be rather weak as compared with that of IAA[8-11]. Cohen and co-workers reported synthesis of a mixture of 5,6- and 4,5-Cl_2-IAAs by Fischer's indolization, but they did not separate the mixture into the isomers[13]. Therefore, the physicochemical properties as well as the biological activities of these two dichloro-IAAs remained unclear.

We have now synthesized all isomers of dichloro-IAAs in a pure form, and measured their auxin activity. As described below, 5,6-Cl_2-IAA (**1**) showed much stronger activity than any of the other dichloro-isomers and of typical auxins such as IAA, 4-Cl-IAA, 2,4-D and NAA.

5,6-Cl_2-IAA (**1**) was synthesized by Fischer's indolization. 3,4-Dichlorophenylhydrazine hydrochloride was coupled with 4,4-dimethoxybutyric acid (prepared from 4,4-dimethoxybutyronitrile by alkaline hydrolysis followed by acidification) in a benzene-water (v/v = 4/1) solution under reflux for 1.5 h to afford the hydrazone in 89% yield. The hydrazone was then subjected to Fischer's indolization by heating at 100 °C with anhydrous zinc chloride in dry xylene for 2 h. This gave a mixture of 5,6- and 4,5-Cl_2-IAAs in 37% yield. The mixture was separated by column chromatography on silica gel using a solvent system of ethyl acetate in *n*-hexane to give pure 5,6- and 4,5-Cl_2-IAAs in a ratio of 5:4. 5,6-Cl_2-IAA: m.p. 189–191 °C; ^{1}H NMR spectrum (acetone-d_6, TMS, ppm) 3.74(2H, doublet, J = 1Hz), 7.40 (1H, broad singlet), 7.60 (1H, singlet), 7.77(1H, singlet); mass spectrum



(1)

Figure 1. The structure of 5,6-dichloroindole-3-acetic acid.

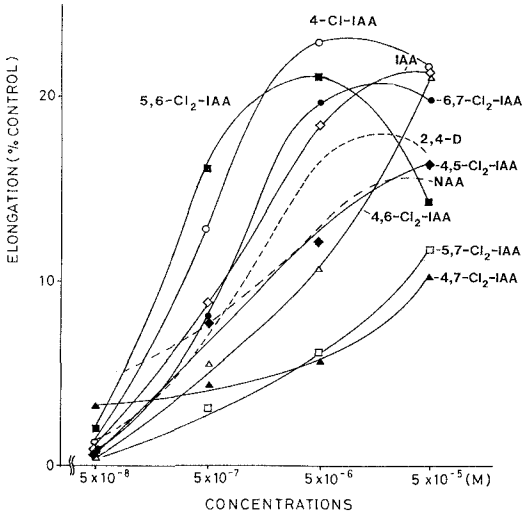


Figure 2. Elongation of *Avena* coleoptiles with dichloro-IAAs, 4-Cl-IAA, IAA, 2,4-D and NAA. Coleoptile segments (5 mm length) that were cut from 2 mm below the tip of seedlings of *Avena sativa* cv. Victory-1, grown under red light for 2 days and then in the dark for 1 day, were incubated in a sample-containing aqueous solution (2 ml) in the dark at 25 °C for 16 h, and the increased length of coleoptiles was measured[16].