racemic hyalodendrin methyl ether (40% of colorless oil) whose identity was established by comparison of IR-, NMR- and mass spectra, as well as TLC behavior with those of an authentic sample. The methyl ether was then cleaved by treatment with boron trichloride in methylene chloride at −70°, affording, after preparative TLC, hyalodendrin (52%) pale yellow crystals from methylene chloride-ether, mp 131–134°, identified by comparison of IR-, NMR-and mass spectra and TLC behavior with those of the natural antibiotic.

Note added in proof. After submission of the manuscript, a report came to our attention describing the isolation of a metabolite with plane structure I, but undetermined absolute configuration, from an unidentified fungus (NRRL 3888) (R. L. DE VAULT and W. ROSENBROOK, J. Antibiot. *26*, 532 (1973)). The enantiomer (3R, 6R) of hyalodendrin, I, has been isolated from *Penicillium turbatum* (K. H. MICHEL, M. O. CHANEY, N. D. JONES, M. M. HOEHN and R. NAGARAJAN, J. Antibiot. *27*, 57 (1974)).

Résumé. L'antibiotique hyalodendrine, produit métabolique d'une espèce d'*Hyalodendron* a été synthétisé en forme racémique utilisant la méthode de Kish

G. M. STRUNZ and M. KAKUSHIM

Canadian Forestry Service,
P.O. Box 4000, Fredericton
(New Brunswick E3B 5G4, Canada),
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Identification of 4-Chloro-4′-hydroxybiphenyl and 4,4′-Dichloro-3-hydroxybiphenyl as Metabolite of 4-Chloro- and 4,4′-Dichlorobiphenyl Fed to Rats

Polychlorinated biphenyls (PCBs) are among the most widespread pollutants in the global environment[1,2]. Recent results have indicated that commercial PCBs (i.e. Aroclor preparations) and individual chlorobiphenyl isomers are susceptible to photolytic degradation with the formation of an array of photoproducts[3–6]. In addition it has also been shown that in a number of plant, animal and microbial systems[7–11] chlorobiphenyl isomers a converted into oxidation products some of which hav been tentatively identified as chlorohydroxybipheny by mass spectrometric analysis. The present report dea with the elucidation of the precise structure of the hydr xylated metabolites of 4-chlorobiphenyl and 4,4′-c chlorobiphenyl fed to rats.

Materials and methods. Male Wistar rats (ca. 75 g bo weight) were fed the isomeric chlorobiphenyl (50 mg/k by injection of this sample in oil every 24 h for 3 day Urine and feces were collected for 1 week from the sta of the feeding experiments. Urine samples were combine diluted with an equal volume of 8 *N* sulphuric acid a refluxed for 1 h. The hydrolyzate was extracted with equal volume of ether (2×) and the combined ether extracts dried, concentrated and purified by thin-lay chromatography. The thin-layer bands were assayed mass spectrometry and those bands containing the chlo hydroxybiphenyl metabolites were treated with ace anhydride-sodium acetate for 1 h at 100°C. The cru acetate fraction was purified by thin-layer chromat graphy and the 220 MHz nuclear magnetic resonan (NMR) spectra obtained on the purified acetoxy meta

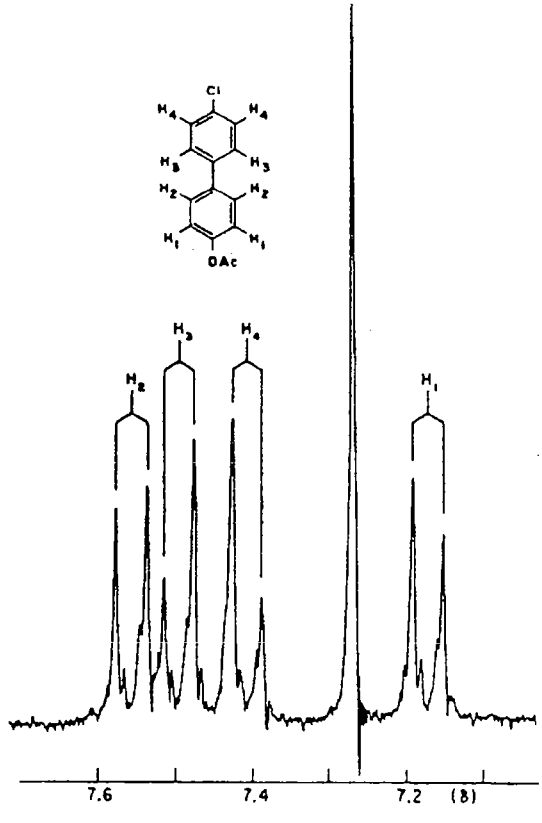

Fig. 1. NMR-spectrum of 4-acetoxy-4′-chlorobiphenyl.

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[11] O. HUTZINGER, S. SAFE, D. ECORICHON, A. S. W. DEFREI R. J. NORSTROM, J. D. MACNEIL, D. J. WILDISH and V. ZI *PCB in the Environment* (Marcel Dekker Publishers, New Y in press.

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olites (ca. 0.5–1.0 mg). The spectra were obtained after several scans using a computer averaging technique.

Results. The NMR-spectra of the acetoxychlorobiphenyl metabolite is shown in Figure 1 and consists of 4 recognizable doublets which is consistent with a symmetrical structure. Thus the hydroxyl group must have been introduced into the 4′ position of the biphenyl nucleus and this structure is consistent with the chemical shifts and coupling constants shown for 4-acetoxy-4-chlorobiphenyl (Figure 1).

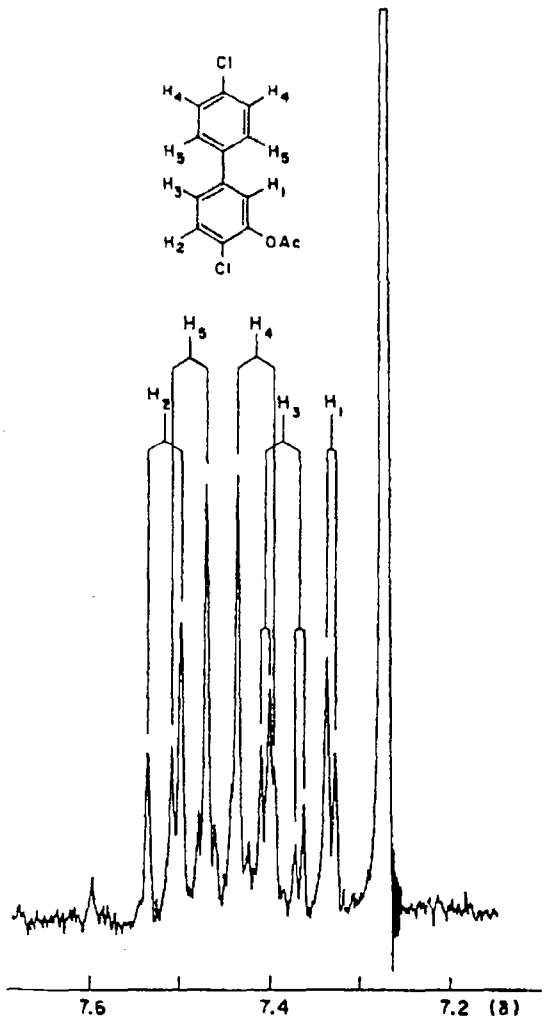

Fig. 2. NMR-spectrum of 3-acetoxy-4,4′-dichlorobiphenyl.

The NMR-spectra of the acetoxydichlorobiphenyl which was formed from feeding 4,4′-dichlorobiphenyl to rats is shown in Figure 2. The set of 2 doublets for the symmetrical H_4 and H_5 protons are easily recognized at δ 7.42 and 7.48 ppm. Since the hydroxyl group can only be introduced at the 2 or 3 position of the biphenyl ring the structure assignments shown in Figure 2 are consistent with hydroxylation at position 3 to give 3-acetoxy-4,4′-dichlorobiphenyl. H_1 is *meta* coupled with H_3; H_3 in turn gives a quartet due to *meta* coupling with H_1 and *ortho* coupling with H_2; H_2 appears as a doublet couplet only with H_3. These coupling constants would also be consistent for 2-acetoxy-4,4′-dichlorobiphenyl even though introduction of an hydroxyl group into the sterically-hindered 2 position would be less likely. The structures of both 4-acetoxy-4-chlorobiphenyl and 3-acetoxy-4,4′-dichlorobiphenyl were confirmed by unambiguous synthesis of the authentic compounds[12] which in turn, were identical to the two metabolites. The mechanism of the hydroxylation and further metabolic degradation of isomeric PCBs are currently under investigation.

Zusammenfassung. Identifizierung und Strukturaufklärung zweier Metaboliten von 4-Chorbiphenyl und 4,4′-Dichlorbiphenyl mittels NMR-Spektroskopie.

S. Safe[13], O. Hutzinger[14] and D. Ecobichon[15]

Department of Chemistry, University of Guelph, Guelph (Ontario, Canada N1G 2W1), Institut für ökologische Chemie, D-8051 Attaching (Federal Republic of Germany), and Department of Pharmacology, Dalhouse University, Halifax (Nova Scotia, Canada),
27 November 1973.

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[13] Department of Chemistry, University of Guelph, Guelph, Ontario, Canada.
[14] Institut für Ökologische Chemie, D-8051, Attaching, W. Germany.
[15] Department of Pharmacology, Dalhouse University, Halifax, Nova Scotia, Canada.

Volatile Ketones in the Hairpencil Secretion of Danaid Butterflies (*Amauris* and *Danaus*)

Chemical studies of the courtship pheromones of butterflies of the subfamily Danaidae[1–4], prompted by earlier behavioral investigation of these insects[5] have led to the isolation of a heterocyclic ketone, 2,3-dihydro-7-methyl-1*H*-pyrrolizin-1-one (I) from the glandular abdominal brushes ('hairpencils') of males of 3 species of the group. In 1 species, the Queen butterfly *Danaus gilippus*, which was studied in detail, the ketone functions as an aphrodisiac, administered by the males in flight to the antennae of the females[6, 7]. The antennae are electrophysiologically sensitive to the ketone[8, 9]. Other danaid butterflies are known[10] or presumed to court in a similar way, although it is now becoming apparent that the chemical repertory of the hairpencils is diversified. Culvenor et al.[11] have reported the isolation of the closely related heterocyclic aldehydes II and III from the males of several species. We here report on the hairpencil chemistry of nine species and subspecies of the genera *Amauris* and

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