APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2005; **19**: 473–478
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.806

Synthesis and characterization of several cephalothin derivatives modified with germanium-containing moieties†

Yoshito Takeuchi*, Yumiko Kase and Yoko Imafuku

Department of Chemistry, Faculty of Science, Kanagawa University, 2946 Tsuchiya, Hiratsuka-shi 259-1293, Japan

Received 23 July 2004; Revised 10 August 2004; Accepted 29 August 2004

A cepham, cephalothin (4), was modified with germanium-containing moieties. Their structures were confirmed chiefly based on electrospray ionization mass spectrometry and NMR spectroscopy and their antibacterial properties were tested. None of these exhibited activity strong enough to be used as a medicine. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: cephalothin; organogermanium compound; ^{1}H NMR; ^{13}C NMR; antibacterial activity

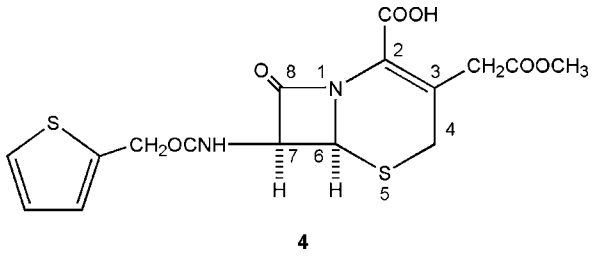
INTRODUCTION

In a previous communication[1] we reported the synthesis, characterization and pharmaceutical assay of three penicillins, i.e. 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (penicillin G) (**1**), 3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (penicillin V) (**2**) and 6-[amino(phenyl)acetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (ampicillin) (**3**) modified with germanium-containing moieties. None of these, however, exhibited activity that was stronger than the parent penicillins.[1]

We considered it important that a similar attempt be made with cephalosporins, which form one of the most important group of β-lactam antibiotics. For this purpose 3-[(acetyloxy)methyl]-8-oxo-7-[(2-thienylacetyl)amino]-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid (cephalothin **4**; Scheme 1) was chosen because it is a widely used commercially available antibiotic.

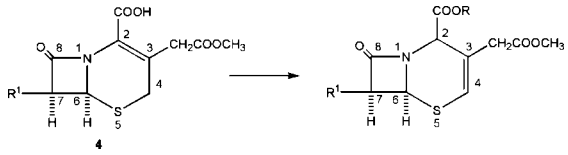
In this paper, an attempt to modify **4** with germanium by esterification of carboxy group is described.

*Correspondence to: Yoshito Takeuchi, Department of Chemistry, Faculty of Science, Kanagawa University, 2946 Tsuchiya, Hiratsuka-shi 259-1293, Japan.
E-mail: yoshito@chem.kanagawa-u.ac.jp
†Dedicated to the memory of Professor Colin Eaborn who made numerous important contributions to the main group chemistry.
Contract/grant sponsor: The Ministry of Education, Culture, Sports, Science and Technology.



4

Scheme 1. Structure of cephalothin.



4

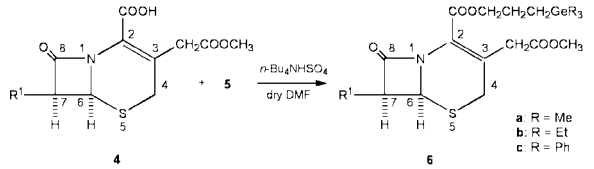
Scheme 2. Isomerization during esterification of cephalothin.

RESULTS AND DISCUSSION

Synthesis

The synthesis of germyl iodides 3-(trimethylgermyl)-1-propyl iodide (**5a**), 3-(triethylgermyl)-1-propyl iodide (**5b**) and 3-(triphenylgermyl)-1-propyl iodide (**5c**) has been described previously.[1] The carboxy group of **4** was reacted with germylpropyl iodides **5** to afford the corresponding germyl esters **6**.[1]

A serious problem accompanying the esterification of **4** is a possible isomerization, e.g. from $^{2}\Delta$ isomer to $^{3}\Delta$ isomer of the esters obtained as depicted in Scheme 2.



Scheme 3. Esterification of cephalothin without isomerization.

It was reported that esterification in the presence of tetra-*n*-butylammonium hydrogensulfate, *n*-Bu_4NHSO_4, substantially reduces (essentially inhibits) isomerization.[2] Hence, we employed this method (Scheme 3).

Three germyl esters, 3-(trimethylgermyl)-1-propyl- (**6a**; 52.2%), 3-(triethylgermyl)-1-propyl (**6b**; 48.0%) and 3-(triphenylgermyl)-1-propyl- (**6c**; 52.5%) of **4** were obtained.

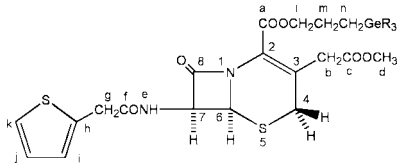
Characterization by electrospray ionization mass spectrometry

Characterization of **6a**–**6c** was based on electrospray ionization mass spectrometry (ESI-MS) in addition to ^1H and ^{13}C NMR spectroscopy.

ESI-MS confirms the formation of germyl esters in two ways. First, it shows a very exact molecular ion peak. For **6a**, the mu of the observed molecular ion peak is 579.06577 which is only different from the calculated mu (579.06548 for $^{12}C_{22}{}^1H_{30}{}^{74}Ge^{14}N_2{}^{23}Na^{16}O_6{}^{32}S_2$ ($[M + Na]^+$) by 0.30 mmu. Second, the splitting pattern of the peak corresponds precisely to the distribution of five germanium isotopes.[3]

NMR spectroscopy

NMR spectroscopy of β-lactam antibiotics has been extensively investigated from the early 1960s. ^1H NMR spectroscopic data of **4** have been reported previously.[4]



Scheme 4. Numbering of atoms of cephalotin derivatives **6a**–**6c**; this numbering applies also to **4**.

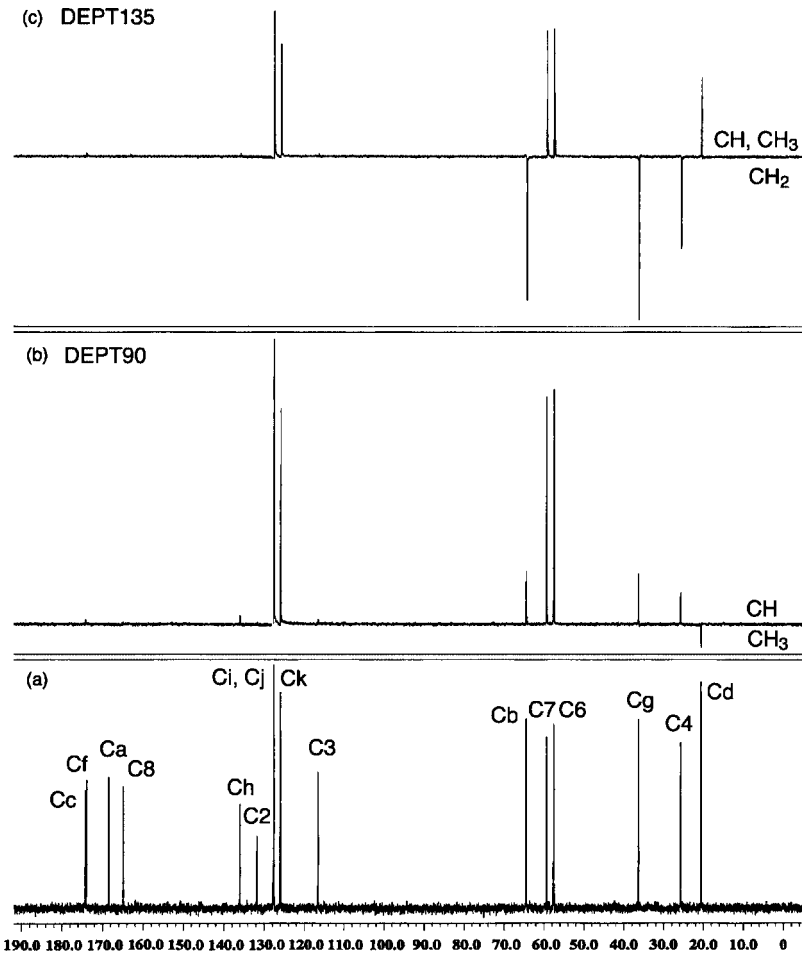


Figure 1. DEPT spectra of **4** (sodium salt): (a) broadband decoupling; (b) DEPT90; (c) DEPT 135.

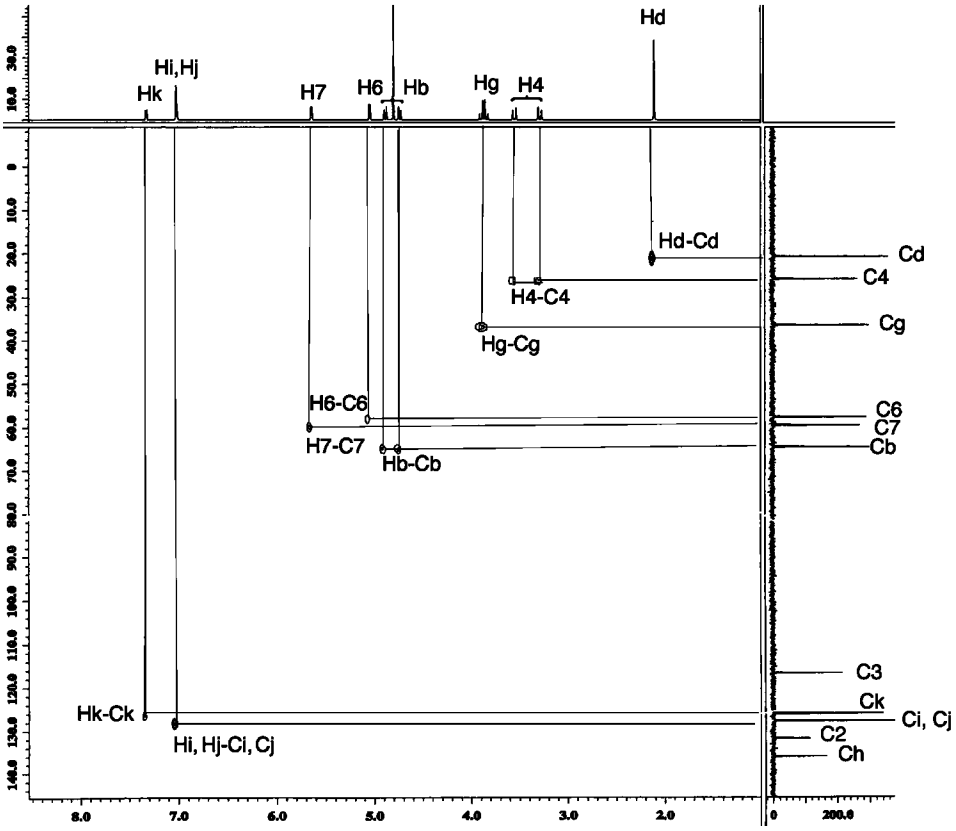


Figure 2. HMQC spectrum of **4** (sodium salt).

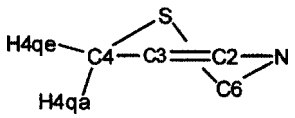


Figure 3. Methylene protons in a cyclohexene moiety.

Assignment of the ^{1}H NMR spectrum of **4** is straightforward, and our own measurements support the assignment by previous workers. There remains one point to be determined, i.e. the differentiation of geminal H4 protons. We could solve this problem with the aid of heteronuclear multiple bond coherence (HMBC) data, which will be described later (Scheme 4). There is no problem in the assignment of the spectra of the germyl esters **6a**– **6c**.

The assignment of the ^{13}C NMR spectrum of **4** has been reported previously.[5] Since the assignment was made chiefly based on the comparison with the data of other cephalosporins, it may be worthwhile making an unequivocal assignment based on modern techniques. In fact, the differentiation between Cc and Cf signals is not clear, and there is one unresolved overlapping of signals Ci and Cj (and hence of signals Hi and Hj).

Figures 1 and 2 give the DEPT and heteronuclear multiple quantum correlation (HMQC) spectra of **4**.

Figures 1 and 2 confirmed the previous assignment of both ^{1}H and ^{13}C spectra of **4**, except for the remaining three uncertainties: (1) differentiation of two H4 protons—one is quasi-axial (qa) and the other quasi-equatorial (qe) (Fig. 3); (2) differentiation of two carbonyl carbon atoms, Cc and Cf; (3) differentiation of two protons and ^{13}C nuclei belonging to the thiophene moiety, i.e. Hi, Hj and Ci, Cj. The remaining assignments (1) and (2) could be solved with the aid of HMBC. The HMBC of **4** is given in Figure 4.

It is noteworthy that the upper field part of the H4 peaks exhibits correlation with both C6 and Cb, whereas the lower field counterpart shows no correlation. Since it is well established that $^{trans}J$(CH) is larger than $^{gauche}J$(CH) in general,[6] and that H4qe is trans and H4qa is gauche to C6, the upper field part of H4 peaks can be assigned to H4qe.

As for differentiation of the two carbonyl peaks at δ 170.13 and 170.29, the H7 peak correlates with the peak at δ 170.13, which can be assigned to Cf (^{3}J(CH)). The other observed correlation is assigned to Hb–Cc (^{2}J(CH)).

The problem with assignment (3) could not be resolved because the overlapping of peaks was so excessive.

^{1}H and ^{13}C NMR parameters of **4** and **6a**–**6c** are summarized in Tables 1 and 2 respectively. The numbering of atoms is given in Scheme 4. Once the assignment of **4** was complete, it was not difficult to make a full assignment of **6a**–**6c**.

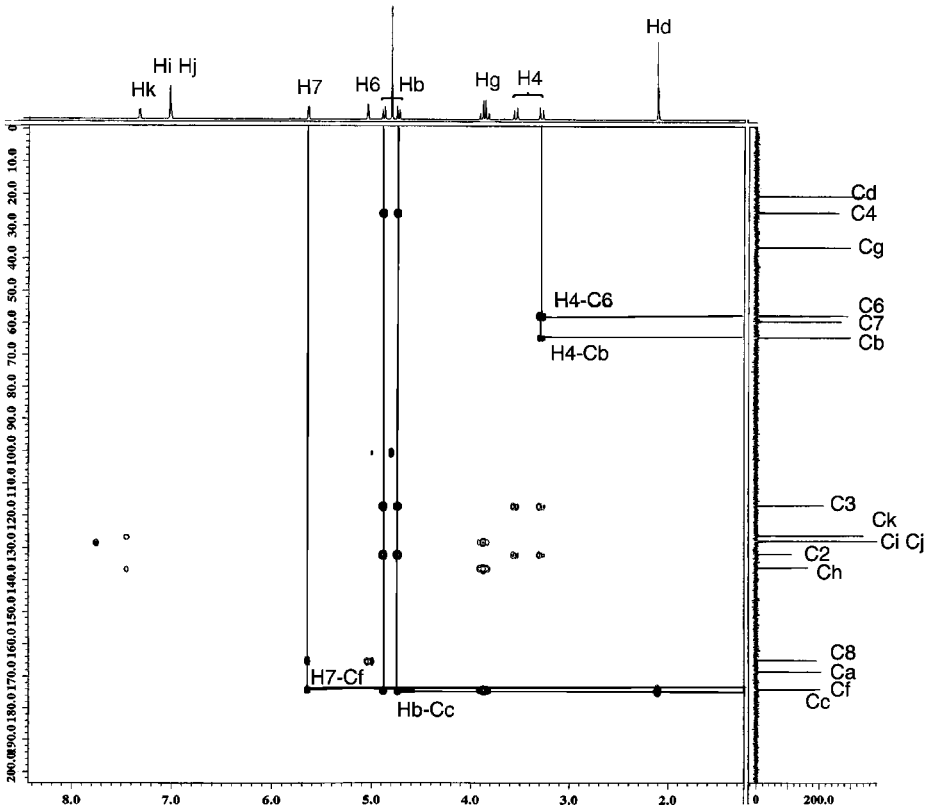


Figure 4. HMBC spectrum of **4** (sodium salt).

Table 1. ^{1}H NMR data of **4** (sodium salt) and **6a**–**6c**[a,b,c]

	4	6a	6b	6c
H4	3.35d, 3.59d (3.37, 3.64)	3.31d, 3.49d	3.35d, 3.54d	3.30d, 3.48d
H6	5.08s (5.12s)	4.90d	4.95d	4.90d
H7	5.69s (5.65s)	5.78c	5.81c	5.78c
Hb	4.76d, 4.89d (4.72, 4.88)	4.76d, 5.06d	4.81d, 5.10d	4.77d, 5.06d
Hd	2.12s (2.11s)	2.02s	2.07s	2.00s
Hg	3.89s (3.92s)	3.77s	3.83s	3.78s
Hi	7.06 (7.06)	6.92	6.56–7.01	6.92–6.95
Hj	7.06 (7.06)	7.04	6.56–7.01	6.92–6.95
Hk	7.37 (7.39)	7.18	7.23	7.19
Hl	—	4.14t	4.18t	4.22
Hm	—	1.69	1.73	1.90
Hn	—	0.64	0.67–0.75	1.54
R	—	0.08s	0.67–0.75, 1.00	7.33–7.35, 7.45–7.47

[a] δ_H (ppm) in D_2O.
[b] Values in parentheses are taken from Ref. 5.
[c] s: singlet; d: doublet.

Pharmacology

Germylated cephalothins **6a**–**6c** were screened for their *in vitro* antibacterial activity and the results compared with the activity of **4**. The study was carried out to evaluate the inherent activity of germylated cephalothins against Gram-positive and Gram-negative microorganisms (testing was carried out by Shionogi Research Laboratories, Osaka, Japan).

The standard streak-plate method was used, and the following parameters were held constant: (i) media composition; (ii) incubation time (18 h); and (iii) incubation temperature (37 °C). The results are given in terms of minimum

Table 2. ^{13}C NMR data of **4** (sodium salt) and **6a**–**6c**[a,b]

	4	6a	6b	6c
C2	131.49 (132.4)	125.51	125.52	125.78
C3	116.30 (117.3)	125.24	125.17	125.63
C4	25.47 (26.4)	26.19	26.28	26.65
C6	57.31 (58.2)	57.24	57.26	57.61
C7	59.12 (60.0)	59.03	59.07	59.43
C8	164.65 (165.5)	161.19	161.08	161.43
Ca	168.22 (169.0)	164.46	164.26	164.63
Cb	64.17 (65.0)	62.88	62.90	63.23
Cc	173.94 (174.8)	170.29	170.18	170.54
Cd	20.35 (21.2)	20.51	20.60	20.94
Cf	173.65 (174.3)	170.13	169.95	170.25
Cg	36.09 (37.0)	36.69	36.82	37.22
Ch	135.74 (136.6)	134.93	134.75	135.08
Ci	127.37/127.42 (128.3)	127.24/127.04	127.06/127.30	127.48/127.72
Cj	127.37/127.42 (128.3)	127.24/127.04	127.06/127.30	127.48/127.72
Ck	125.68 (126.5)	125.39	125.46	125.89
Cl	—	68.45	68.73	68.69
Cm	—	23.83	24.00	24.41
Cn	—	12.05	6.93	10.23
R	—	−2.64	3.69, 8.81	128.39, 129.15, 134.95, 136.60

[a] δ_C (ppm) in D_2O.
[b] Values in parentheses are taken from Ref. 6.

Table 3. Antibacterial sensitivity testing of cephalothins in terms of MIC[a]

Sample	6a	6b	6c	4
Staphylococcus aureus Smith	4	8	>64	0.25
S. aureus SR3637(H-MRSA)	>64	>64	>64	128
Staphylococcus epidermidis ATCC14990	64	32	>64	0.25
S. epidermidis SR25009(MRSE)	>64	>64	>64	64
Streptococcus pneumoniae Type I	8	16	>32	0.125
S. pneumoniae SR16675(PRSP)	>32	>32	>32	8
E faecalis ATTC49757(Bla+)	>64	>64	>64	64
E faecium SR7917(vanA)	>64	>64	>64	>128
E. faecium SR23546	>64	>64	>64	>128
Escherichia coli NIHJ JC-2	>64	>64	>64	8
E. coli SR21003(Toho2)	>64	>64	>64	>128
Klebsiella pneumoniae SR1	>32	>32	>32	2
*K. pneumoniae*ATTC700603(SHV)	>64	>64	>64	>128
Enterobacter cloacae ATCC13047	>64	>64	>64	>128
E. cloacae SR4321 (bla++)	>64	>64	>64	>128
Serratia marcescens ATCC13880	>64	>64	>64	>128
Pseudomonas aeruginosa ATCC25619	>64	>64	>64	>128
P. aeruginosa SR6554(IPM-R)	>32	>32	>32	>128
Haemophilus influenzae ATCC49766	16	>16	>16	1
H. influenzae SR11435(BLNAR)	>16	>16	>16	128
Branhamella catarrhalis ATCC43617(bla+)	16	>32	>32	2

inhibitory concentration (MIC) in Table 3. A larger value indicates that the relevant germylated cephalothin is less effective than **4**. Unfortunately, for bacteria to which cephalothin is effective, none of the germylated esters showed activity, and the germylated esters were also not effective against bacteria to which cephalothin is not effective.

CONCLUSIONS

Initially, we anticipated that selective germylation would be difficult. To our surprise, the reaction proceeded smoothly, and the germylated cephalothins were synthesized in a reasonable yield. NMR spectra indicate that all germylated cephalothins are sufficiently pure for characterization by ESI MS and NMR spectroscopy.

Unfortunately, none of these exhibited activity strong enough to be used as a medicine. This might indicate that, in this case also, the presence of carboxy moieties at C2 will be one of the essential factors for biological activity.

Attempts to synthesize novel antibiotics germylated at the site different from the carboxy function, e.g. transesterification at C3 ester side chain, are in progress in this laboratory.

EXPERIMENTAL

General

^{1}H NMR spectra were determined using a JEOL ECP 500 spectrometer operating at 500 MHz, and ^{13}C NMR spectra were determined with the same spectrometer operating at 125 MHz. In both cases the chemical shifts were reported in δ (ppm) with tetramethylsilane as the internal standard.

ESI mass spectra were recorded with a PerSeptive Biosystems DE MALDI-TOF mass spectrometer, Voyager Elite XL.

Synthesis of germylated cephalothins (6a–6c): general procedure

The synthesis of 3-substitutedgermyl-1-propyl iodide (**5a**–**5c**) was reported previously.[1]

Cephalothin sodium salt (**4**) 0.84 g (2.0 mmol), 0.72 g (2.5 mmol) of **5a** and 1.02 g (3.0 mmol) of *n*-Bu_4 ($AHSO_4$) were dissolved in 25 ml of dry *N*,*N*-dimethyl formamide (DMF). The resulting mixture was stirred under nitrogen atmosphere for 20 h. DMF was removed *in vacuo* and the residue was partitioned with ethyl acetate and water. The organic layer was separated, washed with saturated sodium chloride solution, then dried with sodium sulfate. After concentration and purification by gel-permeation chromatography, 0.29 g (0.52 mmol, 52.2%) of a pure cephalothin ester **6a** was obtained. Syntheses of **6b** and **6c** were carried out in a similar manner.

Acknowledgments

This work was partly supported by the High-Tech Research Center Project from The Ministry of Education, Culture, Sports, Science and Technology. We are grateful to Shionogi Research Laboratories for the determination of the antibacterial activities of the cephalothins prepared.

REFERENCES

1. Imafuku Y, Takeuchi T. *Appl. Organometal. Chem.* **18**: 384.
2. Wang HP, Lee JS. *United States Patent* 5498787, 1996.
3. Suzuki R, Matsumoto T, Tanaka K, Takeuchi Y, Taketomi T. *J. Organometal. Chem.* 2001; **636**: 108.
4. Branch SK, Casy AF, Ominde MA. *J. Pharm. Sci.* 1987; **5**: 73.
5. Tori K, Nishikawa Y, Takeuchi Y. *Tetrahedron Lett.* 1981; **22**: 2793.
6. Marshall JL. *Carbon–Carbon and Carbon–Proton NMR Couplings*. VCH; 1985.