ISOLATION AND CHARACTERIZATION OF FOUR POLYETHER ANTIBIOTICS, X-14889A, B, C, AND D, CLOSELY RELATED TO LYSOCELLIN AND THE FERENSIMYCINS

JOHN W. WESTLEY†, CHAO-MIN LIU, JOHN F. BLOUNT, LOUIS TODARO, LILLIAN H. SELLO and NELSON TROUPE†

Roche Research Center, Hoffmann-La Roche Inc.,
Nutley, NJ 07110, U.S.A.
†Smithkline and Beecham Pharmaceuticals,
P.O. Box 1539, King of Prussia, PA 19406-0939, U.S.A.

(Received for publication September 30, 1992)

Streptomyces sp. X-14889 (NRRL 15517) has been found to produce a number of novel polyether antibiotics and an orange pigment. One of the antibiotics, X-14889B (**3**) was identified as ferensimycin A, which in turn is an isomer of the well-studied polyether antibiotic, lysocellin (**1**). Of the three other antibiotics, X-14889C (**4**) is a lower homolog of ferensimycin A and antibiotics X-14889A (**2**) and D (**5**) which are respectively the descarboxy and anhydro-descarboxy forms of this same molecule. The latter compound, X-14889D is of interest as it contains an ether bridge across the terminal tetrahydrofuranyl ring in an analogous relationship to that reported earlier for antibiotics X-14873A (**6**) and G.

In 1982, KUSAKABE *et al.*[1] reported the isolation of two novel polyether antibiotics, ferensimycins A and B. Ferensimycin A is an isomer of the commonly occurring polyether antibiotic, lysocellin[2] and belongs to the same basic structural type as the recently reported polyether complex of antibiotics X-14873A, G and H[3]. In this paper, a further complex of this type is reported. In fact, one component of the complex, antibiotic X-14889B (**3**) has been shown to be identical to ferensimycin A. Antibiotic X-14889C (**4**) is a lower homolog of ferensimycin and the other two components of the complex, X-14889A (**2**) and D (**5**) are respectively the descarboxy and the anhydro-descarboxy derivatives of X-14889C. In addition to these four isolated polyethers, an orange pigment (**7**) was also isolated from *Streptomyces* X-14889 (NRRL 15517) and characterized.

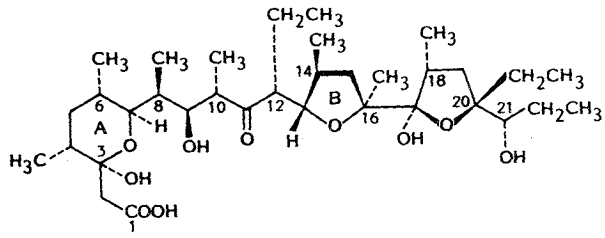
The antibiotic complex was isolated from the fermentation broth by ethyl acetate extraction and purified by silica gel chromatography. The antibiotics are mainly active against Gram-positive bacteria and exhibit ionophoretic activity. Antibiotic X-14889C is also effective in stimulating the production of propionate in a rumen fermentation system[4].

Isolation of Antibiotics X-14889A, B, C and D

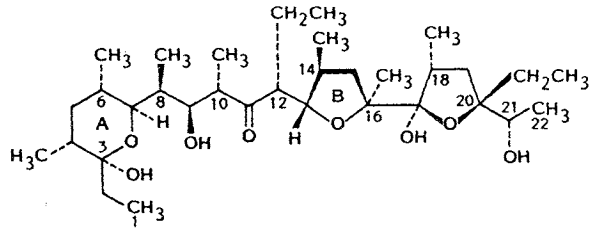
As part of our search for novel polyether antibiotics with exceptional activity either as coccidiostats or ruminant growth promotants[5], the whole broth from a fermentation of *Streptomyces* sp. X-14889 (NRRL 15517) was extracted twice with equal volumes of ethyl acetate and the extract subjected to a series of partition and chromatography steps described in detail elsewhere[5]. Antibiotic X-14889A was isolated as a crystalline solid from hexane, mp 149~150°C, $[\alpha]_D$ 29.3° (*c* 1, MeOH), 6.7° (*c* 1, $CHCl_3$). Microanalysis calcd for $C_{33}H_{60}O_8$ (584.84): C 67.77, H 10.34. Found: C 67.78, H 10.50.

From the same column chromatography that yielded antibiotic X-14889A, a second fraction was shown to contain antibiotic X-14889B, which crystallized as the hemihydrated sodium salt from diethyl

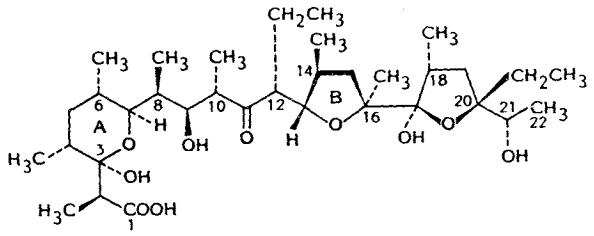
The structures of lysocellin, antibiotic X-14873A, antibiotics X-14889A, B, C and D.



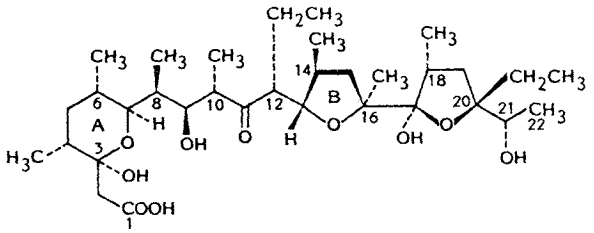
Lysocellin (**1**)



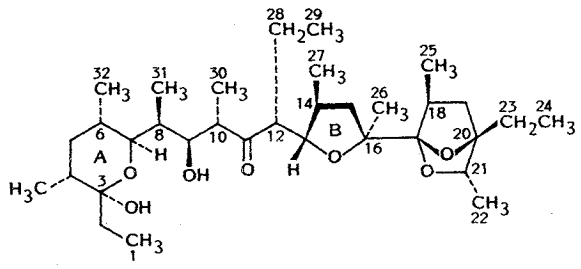
X-14889A (**2**)



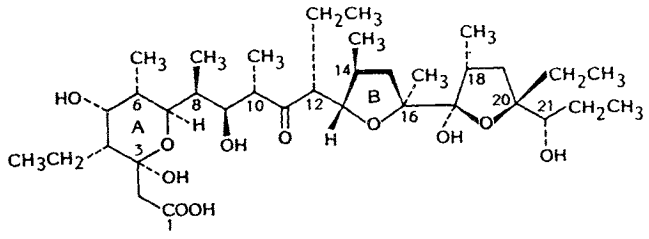
X-14889B (**3**)



X-14889C (**4**)

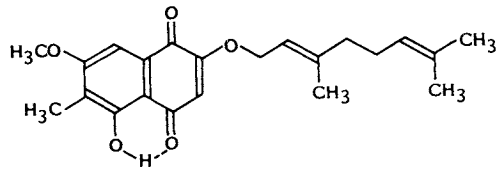


X-14889D (**5**)



X-14873A (**6**)

(Continued)



7

1,4-Dihydro-5-hydroxy-7-methoxy-6-methyl-2-[(3,7-dimethyl-2,6-octadienyl)oxy]-1,4-naphthalenedione.

Fig. 1. Stereoscopic drawings of the sodium salt of antibiotic X-14889C (above) and the thallium salt of X-14889B (below).

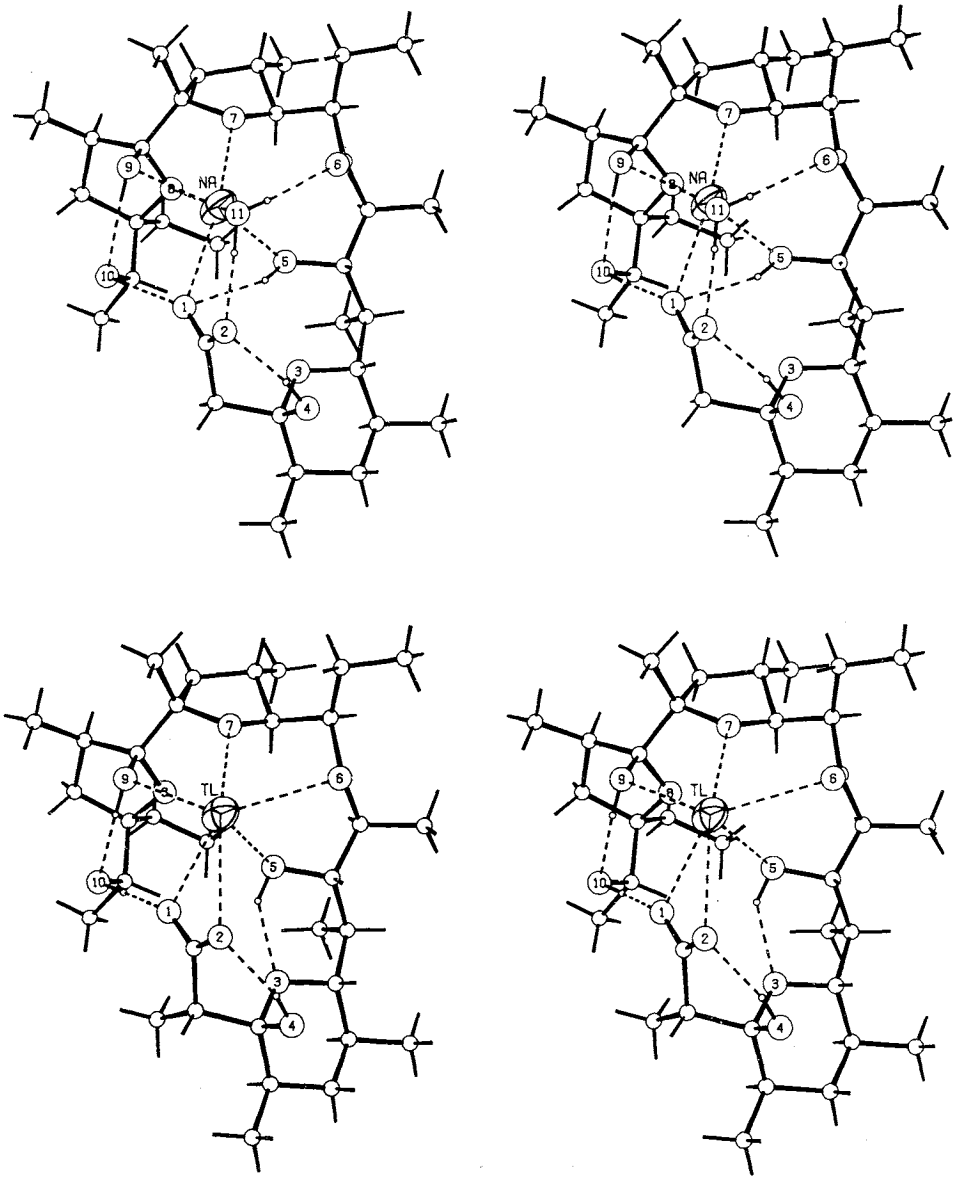


Table 1. Coordination of the thallium ion by X-14889B.

Oxygen	Distance (Å) Tl...O
O (1)	2.686
O (5)	2.781
O (9)	2.856
O (2)	2.934
O (6)	2.942
O (7)	2.952

Table 2. Coordination of the sodium ion by X-14889C.

Oxygen	Distance (Å) Na...O
O (11)	2.296
O (5)	2.356
O (7)	2.411
O (9)	2.413
O (1)	2.490
O (8)	2.786
O (6)	(3.014)

Table 3. Hydrogen bonds in the thallium salt of X-14889B.

Hydrogen bond	O...O Distance (Å)
O (4)–H.....O (2)	2.66
O (5).....O (1)	2.98
O (5)–H.....O (3)	2.74
O (9)–H.....O (10)	2.80
O (10)–H.....O (1)	2.63

Table 4. Hydrogen bonds in the sodium salt of X-14889C.

Hydrogen bond	O...O Distance (Å)
O (4)–H.....O (2)	2.66
O (5)–H.....O (1)	2.97
O (5).....O (3)	2.72
O (9)–H.....O (10)	2.82
O (10)–H.....O (1)	2.63
O (11)–H.....O (2)	2.71
O (11)–H.....O (6)	3.10

ether, mp 139~140°C, $[\alpha]_D$ 5.9° (*c* 1, MeOH), 7.4° (*c* 1, $CHCl_3$). Microanalysis calcd for $C_{34}H_{59}O_{10}Na \cdot 5H_2O$ (659.84): C 61.89, H 9.17, Na 3.48 and H_2O 1.37. Found: C 61.92, H 9.27, Na 3.55, H_2O 1.71.

The structure of antibiotic X-14889B was determined by X-ray analysis of its thallium salt and was later reported in the literature under the name ferensimycin A by KUSAKABE *et al.*[1]

A third fraction from the same column that yielded antibiotic X-14889A and ferensimycin A, gave the sodium salt of antibiotic X-14889C which crystallized from diethyl ether by the addition of hexane as a monohydrate, mp 139~141°C, $[\alpha]_D$ 19.3° (*c* 1, $CHCl_3$). Microanalysis calcd for $C_{33}H_{57}O_{10}Na \cdot H_2O$ (654.82): C 60.53, H 9.08, Na 3.51, H_2O 2.75. Found: C 60.99, H 9.16, Na 3.68, H_2O 2.00.

The structure of X-14889C was determined by X-ray analysis of this hydrated sodium salt, as described in the next section. Rechromatography of the mother liquor of antibiotic X-14889C yielded the fourth member of the complex, antibiotic X-14889D, mp 117°C. Micronalaysis calcd for $C_{33}H_{58}O_7$ (566.82): C 69.93, H 10.31. Found: C 69.60, 69.87, H 10.36, 10.28.

The structure of antibiotic X-14889D was determined by X-ray analysis as described below.

Structure Determination of Antibiotics X-14889B and C by X-Ray Analysis

The structure and absolute configuration of antibiotic X-14889B (ferensimycin A) were determined from a single-crystal X-ray analysis of its thallium salt and the structure of antibiotic X-14889C was determined from an analysis of its sodium salt. Because the latter only differs from ferensimycin A at C-2, the absolute stereochemistry is presumed to be the same.

Drawings of the sodium salt of antibiotic X-14889C and the thallium salt of antibiotic X-14889B are shown in Fig. 1. Careful stereoscopic viewing of the drawings show that the sodium ion is significantly closer to the center of X-14889C than in the case of the thallium ion in antibiotic X-14889B. It can also be observed that the water molecule (O-11) present in the hydrated sodium salt is hydrogen bonded to O-2 and O-6, atoms which participate in the coordination of the thallium ion in X-14889B but not the

Table 5. Torsion angles for antibiotic X-14889B and the salts of X-14889C and D with standard deviation in parentheses.

	X-14889B Tl salt	X-14889C Na salt	X-14889D
C (1)–C (2)–C (3)–O (3)	−54.7 (7)	−61.4 (5)	61.5 (5)
C (2)–C (3)–O (3)–C (7)	173.9 (6)	175.6 (4)	175.2 (3)
C (3)–O (3)–C (7)–C (8)	−171.6 (6)	−172.6 (4)	−170.8 (3)
O (3)–C (7)–C (8)–C (9)	58.2 (7)	57.2 (5)	57.4 (4)
C (7)–C (8)–C (9)–C (10)	159.2 (6)	158.8 (4)	163.7 (4)
C (8)–C (9)–C (10)–C (11)	179.0 (6)	−177.6 (4)	−180.0 (5)
C (9)–C (10)–C (11)–C (12)	−141.2 (7)	−144.2 (4)	−102.8 (4)
C (10)–C (11)–C (12)–C (13)	79.9 (8)	85.3 (5)	151.2 (4)
C (11)–C (12)–C (13)–O (7)	65.4 (8)	62.4 (4)	173.9 (3)
C (12)–C (13)–O (7)–C (16)	155.3 (6)	154.8 (4)	156.8 (3)
C (13)–O (7)–C (16)–C (17)	109.3 (7)	112.9 (4)	110.9 (4)
O (7)–C (16)–C (17)–O (6)	−53.8 (9)	−50.2 (5)	−173.6 (4)
C (16)–C (17)–O (8)–C (20)	−148.5 (8)	−149.3 (4)	172.3 (4)
C (17)–O (8)–C (20)–C (21)	−108.6 (8)	−109.5 (4)	−55.1 (5)
O (8)–C (20)–C (21)–C (22)	−178.0 (10)	−175.4 (5)	159.9 (6)

Table 6. Crystal data for the thallium salt of X-14889B and the sodium salt of X-14889C and X-14889D.

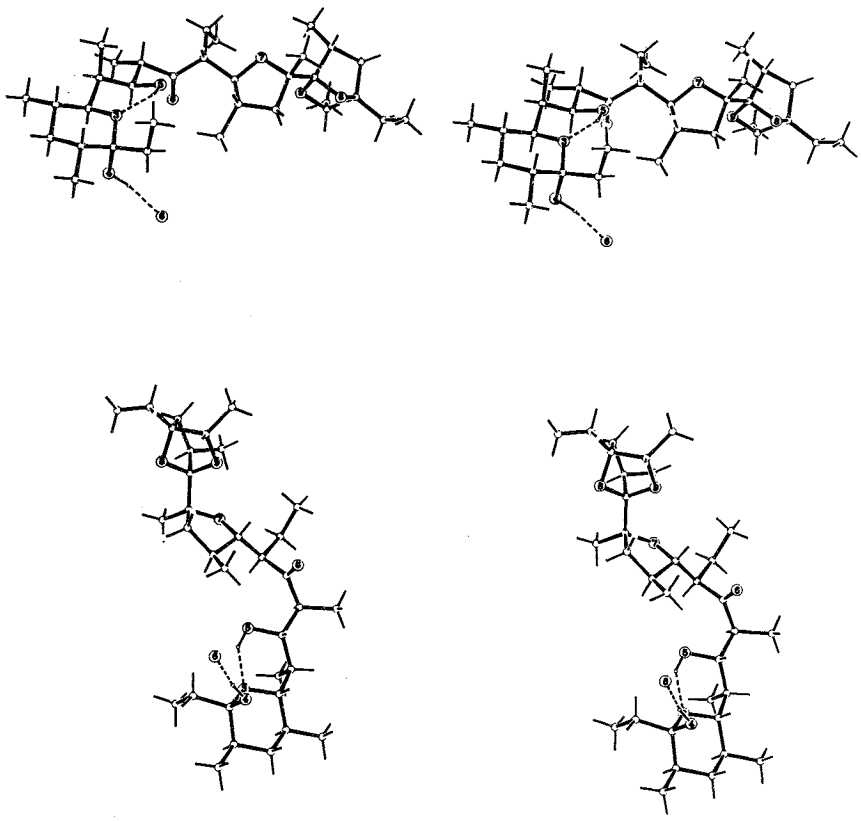
	X-14889B	X-14889C	X-14889D
Formula	$C_{34}H_{59}O_{10}Tl$	$C_{33}H_{57}O_{10}Na \cdot H_2O$	$C_{33}H_{58}O_7$
Formula weight	832.21	654.82	566.82
Space group	$P2_1$	$P2_1$	$P2_12_12_1$
a (A)	9.512 (2)	9.426 (2)	8.931 (2) A
b (A)	10.737 (2)	10.583 (2)	16.229 (2) A
c (A)	18.328 (3)	18.639 (2)	23.900 (4) A
β (°)	90.32 (2)	92.11 (1)	
Z	2	2	4
d Calcd ($g\,cm^{-3}$)	1.476	1.170	1.087
μ (CuKα) (cm^{-1})	87.8	8.2	6.0

sodium ion in X-14889C. The coordination of the cations in both salts are described in Tables 1 and 2, and the hydrogen bonds in the two salts are given in Tables 3 and 4. It is clear from Fig. 1 that the conformation of the two antibiotic molecules are very similar and perusal of the torsion angles in Table 5 confirms this observation. The methyl substituent in X-14889B at C-2 appears to have negligible effect on the conformation of the molecule.

The crystal data for the two salts are given in Table 6. The intensity data were measured on Hilger-Watts diffractometers (Ni filtered CuKα radiation, $\theta-2\theta$ scans, pulse height discrimination). The structures were solved in an unorthodox, but fully effective fashion. As soon as the unit cell for the thallium salt of X-14889B was known, it was immediately clear that it was similar to the previously reported thallium salt of X-14873A[3]. The complete set of non-hydrogen atoms for X-14873A was taken as the starting trial structure for X-14889B and isotopic least squares refinement was begun directly. After six cycles of least squares, the atoms not present in X-14889B were identified by their very high temperature factors and a difference map was calculated to locate any new atoms. The analysis then continued normally. The structure of X-14889C was obtained in a similar way with X-14889B as the starting point.

Fig. 2. Two stereoscopic views of antibiotic X-14889D showing its conformation in the crystalline state.

Lack of a carboxyl group results in loss of the cyclic structures observed for X-14889B and C as illustrated in Fig. 1.



The absolute configuration of X-14889B is based on the anomalous scattering of the thallium atom. The weighted discrepancy index for the configuration indicated was 0.0388 while that for the enantiomeric structure was 0.0502, thus establishing the former as the absolute configuration of the molecule.

Structure Determination of Antibiotic X-14889D

The structure of antibiotic X-14889D has also been determined from a single crystal X-ray analysis. Whereas the differences in structure and conformation discussed for antibiotics X-14889B and X-14889C were quite subtle, the lack of a carboxyl group and the presence of a bridged 1,3-dioxolane ring system clearly distinguish the structure of X-14889D and cause dramatic changes in the conformation of the molecule as illustrated in Fig. 2.

The torsion angles in the antibiotic X-14889D are listed together with those of X-14889B and C in Table 5. Perusal of the data shows that the major conformational changes occur about C(10)–C(11), C(11)–C(12), C(12)–C(13) and C(16)–C(17) bonds. The crystal data are summarized in Table 6. The intensity data were measured as for the other members of the complex and the size of the crystal used for the data collection was $0.12 \times 0.15 \times 0.55$ mm. The structure was solved by a multiple solution procedure[7] and was refined by full matrix least squares. In the final refinement, anisotropic thermal parameters were used for the non-hydrogen atoms and isotropic temperature factors were used for the hydrogen atoms.

The final discrepancy factors were R=0.045 and WR=0.048 for the 2,169 observed reflections.

The structure of antibiotic X-14889A (**2**) was clear from microanalysis, mass spectrometry and nuclear magnetic resonance comparison with X-14889B (**3**), X-14889C (**4**) and X-14889D (**5**).

Finally, an orange pigment **7** isolated from *Streptomyces* X-14889 has been structurally assigned by spectroscopic comparisons with 6-ethyl-5-hydroxy-2,7-dimethoxy-naphthoquinone[8] isolated from *Hendersonula toruloidea* and 2,7-dimethoxy-5-hydroxynaphthoquinone[9] produced by a strain of *Streptomyces*.

References

1) KUSAKABE, Y.; T. MIZUNO, S. KAWABATA, S. TANJI, A. SEINO, H. SETO & N. ŌTAKE: Ferensimycins A and B, two polyether antibiotics. Taxonomy, fermentation, isolation, characterization and structural studies. J. Antibiotics 35: 1119~1129, 1982
2) EBATA, E.; H. KASAHARA, K. SEKINE & Y. INOUE: Lysocellin, a new polyether antibiotic. I. Isolation, purification, physico-chemical and biological properties. J. Antibiotics 28: 118~121, 1975
3) WESTLEY, J. W.; C.-M. LIU, J. F. BLOUNT, L. TODARO, L. H. SELLO & N. TROUPE: Isolation and characterization of three novel polyether antibiotics and three actinomycins as cometabolites of the same *Streptomyces* sp. X-14873, ATCC 31679. J. Antibiotics 39: 1704~1711, 1986
4) LIU, C.-M.; J. W. WESTLEY, J. CHU, T. E. HERMANN, M. LIU & N. J. PALLERONI: Three novel polyether antibiotics X-14889A, C, and D from a streptomycete. Taxonomy of the producing organism, fermentation production and biological properties of the antibiotics. J. Antibiotics 46: 275~279, 1993
5) RUFF, M. D.: Verternary application. *In* Polyether Antibiotics: Naturally Occurring Acid Ionophores. Vol. 1, Biology. *Ed.* J. W. WESTLEY, pp. 303~332, Marcel Dekker, Inc., New York, 1982
6) LIU, C.-M. & J. W. WESTLEY (Hoffmann-La Roche): Antibiotics X-14889A, C & D. U.S. 4,537,956, Aug. 27, 1985
7) GERMAIN, G.; P. MAIN & M. M. WOOLFSON: Applications of phase relationships to complex structures 3 optimum uses of phase relationships. Acta Cryst. A27: 368, 1971
8) HOWE, R. & R. H. MOORE: 6-Ethyl-5 hydroxy-2,7-dimethyloxynaphthoquinone a metabolite of *Hendersonula torulodea nattress*. Experimentia 25: 474, 1969
9) GREBER, N. N. & B. WIECLAW: Applications of phase relationships to complex structures 3 optimum use of phase relationships. J. Org. Chem. 31: 1496, 1966