Tetrahedron Vol. 35, pp. 1035 to 1039
© Pergamon Press Ltd., 1979. Printed in Great Britain

0040-4020/79/0501-1035/$02.00/0

TERPENOIDS—LXXVI[1]

PRECAPNELLADIENE, A POSSIBLE BIOSYNTHETIC PRECURSOR OF THE CAPNELLANE SKELETON

ESER AYANOGLU, TARAKEGN GEBREYESUS, CURTIS M. BEECHAN and CARL DJERASSI*
Department of Chemistry, Stanford University, Stanford, CA 94305, U.S.A.

(*Received in the USA 5 July 1978*)

Abstract—A new sesquiterpene hydrocarbon, precapnelladiene (I) which does not obey the isoprene rule, was isolated from the nonpolar fractions of the soft coral *Capnella imbricata*. Its structure was established by chemical as well as spectral evidence, with additional aid of the CONGEN computer program. Precapnelladiene has a fused 5- and 8-membered ring system and may be a biogenetic precursor of the unique tricyclic capnellane skeleton typical of sesquiterpenes isolated from the same marine animal.

Organic chemical structure elucidation is generally based on a combination of chemical and spectroscopic evidence. Intuition, mostly originating from chemical knowledge and experience, also plays a significant role. In the case of natural products, for characterization of new structures, "biogenetic considerations" are also frequently utilized such as possible biosynthetic pathways, co-occurring compounds, biogenetic analogy, and selection from among known skeletons of those which best fit available data.

In this study, the CONGEN computer program[2] has been used as an adjunct to the spectral and chemical data for the structure elucidation of a new sesquiterpene hydrocarbon isolated from the soft coral *Capnella imbricata*. CONGEN (for CONstrained structure GENeration) is an artificial intelligence technique which has already been utilized for various problems including the generation of possible structures and isomers in organic chemistry.[2-4]

RESULTS AND DISCUSSION

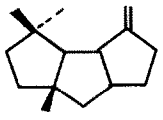
The soft coral *Capnella imbricata* contains a new group of sesquiterpene alcohols[5-8] with a hitherto unknown 5-membered tricyclic skeleton named capnellane (VI). The new hydrocarbon, named precapnelladiene (I), was isolated with some other sesquiterpene hydrocarbons from the nonpolar extracts of *Capnella imbricata* using column chromatography and preparative glc techniques. The structure elucidation of one of these hydrocarbons—$\Delta^{9(12)}$-capnellene (II)—has been reported in a previous paper.[1]

The new hydrocarbon was present in the same nonpolar fractions from which $\Delta^{9(12)}$-capnellene (II) was isolated. It is unstable and slightly volatile at room temperature. Decomposition was partly prevented by keeping a pentane solution of the compound under argon in a freezer. It has a slightly longer glc retention time than that of $\Delta^{9(12)}$-capnellene (II) but the hydrocarbon mixture gave only one spot upon tlc separation. The mass spectrum of the compound showed a molecular ion peak at *m/e* 204. The absence of OH or oxo groups is evident from the IR spectrum and taken together with the high glc mobility leads to the molecular formula $C_{15}H_{24}$.

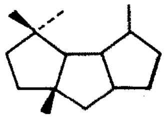
The IR spectrum showed an absorption band at 899 cm^{-1} as a doublet which indicates the presence of a gem-dimethyl or isopropyl group. The 100 MHz NMR spectrum indicated two double bonds and four Me groups. One of the double bond protons was isolated (5.06 ppm (δ), s, 1H) while the other double bond proton signal (5.34 ppm, t, 1H) was split by two protons. The lack of UV absorption indicated that these double bonds are not conjugated. The nature of the Me groups could be defined as follows: one vinyl Me with no proton (1.64 ppm, s, 3H); one secondary Me (1.03 ppm, d, J 5.2, 3H); and one gem-diMe group (0.96 ppm, s, 6H). No isopropyl or cyclopropyl group absorptions were observed.

The molecular formula ($C_{15}H_{24}$) together with the presence of two double bonds and two degrees of unsaturation requires a bicyclic ring system. The absorption around 3.5 ppm hints at the presence of an allylic proton and 4- or 5-membered ring(s).

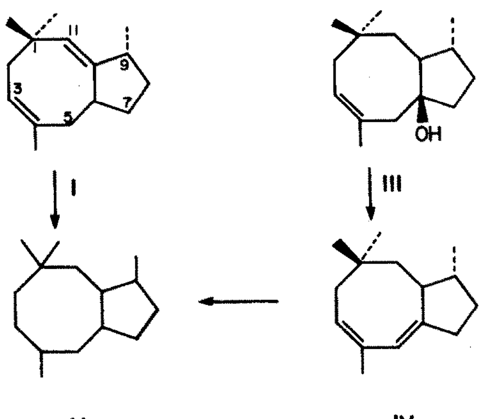
As mentioned earlier the same soft coral contains a hydrocarbon (II) and five alcohols based on the capnellane skeleton (VI). This skeleton also contains one gem-diMe and two other Me groups together with 5-membered rings. These similarities suggest the possibility of a biosynthetic relationship between I and sesquiterpenes derived from VI.



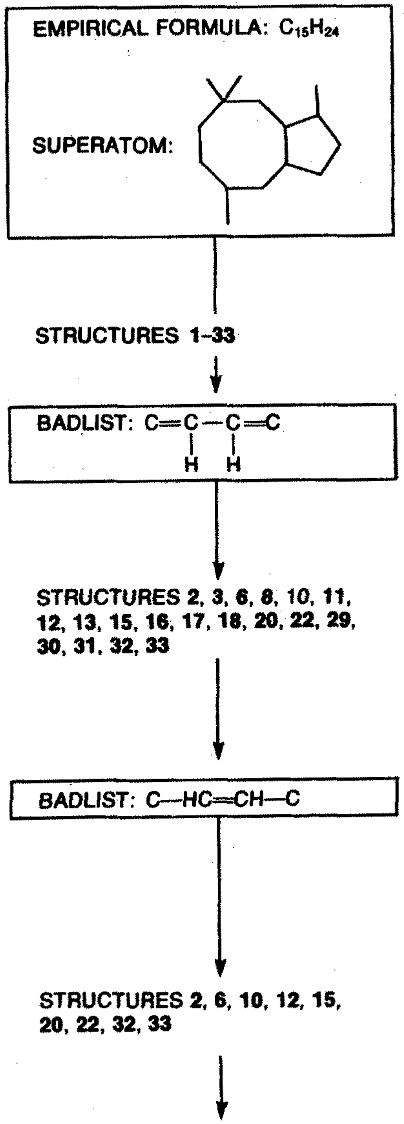
II

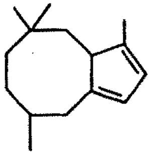


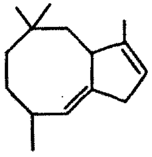
VI

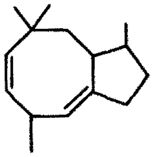


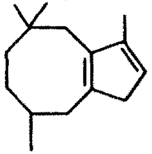
V IV

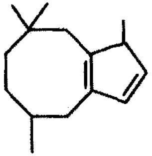


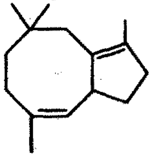

1

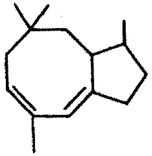

2

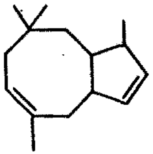

3

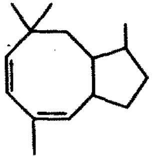

4

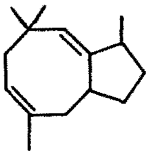

5

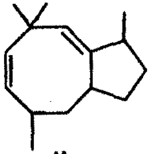

6

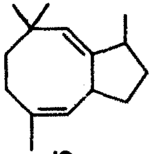

7

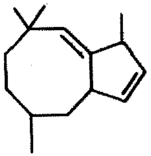

8

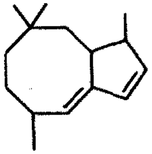

9

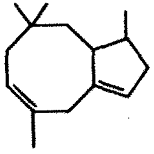

10

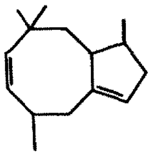

11

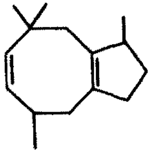

12

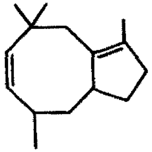

13


14


15


16


17


18

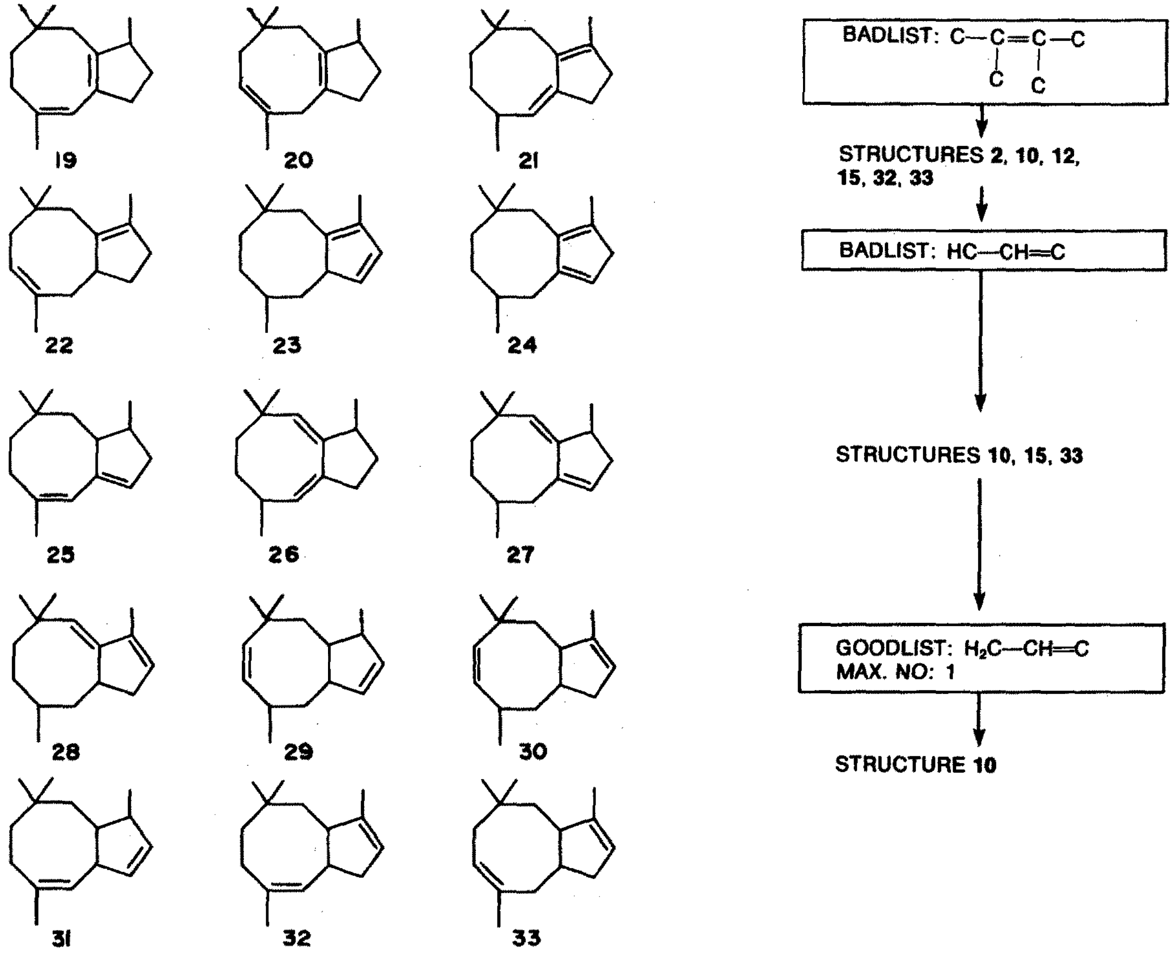


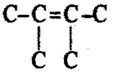
Fig. 1. CONGEN output using precapnelladiene data.

An example of a 5- and 8-membered ring system is dactylol (III) which was isolated from the sea hare *Aplysia dactylomela*.[9] Comparison showed that the NMR spectrum of I is quite similar (Table 1) to that of dactylol except for some $-CH_2$ and $-CH$ absorption differences and a lack of the isolated double bond proton signal for dactylol (5.06 ppm). When dactylol (III) was subjected to $POCl_3$/pyridine dehydration a mixture of hydrocarbons was obtained whose NMR spectrum was similar to that of I. The dehydration mixture upon glc separation gave a broad peak with a shoulder whose retention time was very close to that of I. The presence of conjugated isomer IV in the mixture is evident from the UV absorption at 232 nm. The NMR spectrum of the mixture exhibited a quartet at 3.52 ppm possibly due to the bridgehead proton of IV at C-10 which is adjacent to a double bond and split by three protons. The presence in the natural hydrocarbon I of a quintet at almost the same place (3.54 ppm) suggests that this may be due to the bridgehead hydrogen at C-6 which is also adjacent to a double bond.

Hydrogenation of I under mild conditions gave a mixture of stereoisomers. The dehydrated dactylol mixture without further purification was also subjected to the same hydrogenation conditions and a similar hydrocarbon mixture was obtained. The major products of these two hydrogenation mixtures were separated from the other stereoisomers using preparative high pressure liquid chromatography (HPLC) and preparative glc respectively. Coinjection of these two purified hydrogenation products gave one peak on glc; furthermore they had the same retention time on HPLC as well as identical NMR and mass spectra. These interconversions demonstrate that I must contain the nuclear skeleton V together with two non-conjugated double bonds.

The isolated olefinic proton should be at C-11, because it is adjacent to the tetra-substituted carbon (C-1). Since the other double bond is not conjugated and gives a triplet, it should be at C-3 rather than C-5 (only one neighboring proton).

While it is appreciated that this is a problem in which manual procedures can be used to examine all possible alternatives, we considered it instructive to check the conclusions through the use of the CONGEN program.

The program was given the empirical formula $C_{15}H_{24}$ and skeleton V was listed as a superatom.[2] The values 5 and 8 were entered as GOODRINGS (desired ring sizes) while formation of 3, 4, 6 and 7 membered rings was excluded by entering these numbers as BADRINGS (undesired ring sizes). Without any other constraints 33 possible structures were obtained (Fig. 1). The lack of conjugation (cf. UV spectrum) allowed us to define C=C–C=C as a BADLIST (undesired substructures) constraint, thus reducing the number of structures to 19 (see Fig. 1). The absence of C–HC=CH–C (no doublets in the NMR spectrum double bond area) and the absence of  $C-\underset{\displaystyle C}{\underset{|}{C}}=\underset{\displaystyle C}{\underset{|}{C}}-C$ (I has two double bonds and both of them have one proton) and finally the absence of HC–CH=C (NMR shows no doublets in double bond region) were implemented by entering these substructures as BADLIST constraints. These restrictions decreased the number of possible structures to 9, 6 and 3 respectively (see Fig. 1). As a last step a GOODLIST (names and numbers of desired substructures) entry was made indicating the existence of one double bond proton which is split by two hydrogens (the triplet at 5.34 ppm), thus leaving only one possible structure (10 = I) for precapnelladiene. Based upon strain considerations, both double bonds should possess the *cis* orientation.[10]

As indicated earlier, precapnelladiene (I) may well be the key biosynthetic precursor for all of the tricyclic capnellanes in the soft coral. Its own unusual skeleton may arise in the same manner as that of dactylol (III)[9] but it remains to be seen whether these biosynthetic steps are performed by the soft coral or are carried out partly or totally by some other constituents of its food chain.

EXPERIMENTAL

General. The CONGEN program runs on a Digital Equipment Corporation KI-10 computer at the *S*tanford *U*niversity *M*edical *EX*perimental (SUMEX) computer facility. Structure generation and the user interface parts of the CONGEN are written in the program language INTERLISP. Imbedding and related parts are in SAIL. FORTRAN has been used for the structure drawing part of CONGEN. A copy of the CONGEN documentation which provides more information is available to interested persons. The CONGEN program is also available via a nationwide computer network; the authors should be contacted for additional information.

Analytical and preparative glc were carried out on a Hewlett–Packard 402A chromatograph equipped with a standard 402 flame ionization detector. 4 mm i.d. × 1.5 m "U" shaped glass columns were used for analytical purposes while the internal dia. was 10 mm for preparative analysis. He was used as carrier gas at a flow rate of 100–120 ml/min. Adsorbants packed for various purposes are given below. TLC separations were performed on 5 × 20 cm glass plates, using 250 μ silica gel (E. Merck, HF-254 + 366, type 60) impregnated with 12% $AgNO_3$. Visualization was effected by spraying with a 2% ceric sulfate soln in 1 M H_2SO_4 followed by heating.

Preparative HPLC was performed using a Haskel model 28303 pump, a 0–5000 psi Ashrof gauge, a Waters dual cell refractometer, and a Whatman Partisil M9 1/50 ODS-2 column (50 cm × 8 mm i.d.).

Low resolution GC/MS was performed on a Varian MAT-44 GC/MS system using a 2.7 mm i.d. × 2 m spiral glass column, containing 3% SP-225~~0 on~~ Supercoport 100/200 (Supelco Inc.) with an oven temp. of 110°. IR spectra were obtained on a Perkin–Elmer 421 instrument and UV absorptions on a Cary 14 UV spectrometer. The 100 MHz spectra were run on a Varian Associates XL-100 MHz NMR spectrometer equipped with a NTCFT computer for data acquisition and for Fourier transform processing while a Bruker HXS 360 MHz spectrometer was used for the 360 MHz spectra. Shift values are reported in ppm.

Isolation and purification of precapnelladiene (I). The animal (*Capnella imbricata*, Quoy and Gaimard, 1833, Coelenterata, Anthozoa, Alcyonaria, Alcyonacea) was sun dried, and blended with pentane in a Waring blendor. The filtered solid material was extracted with pentane for 24 hr. The pentane filtrate and ex-

Table 1. Some NMR data of precapnelladiene and dactylol

COMPOUND	$=C\underline{H}-CH_2$	$=C-CH_3$	$CH-C\underline{H}_3$	$C-(CH_3)_2$
Precapnelladiene (I)	5.34 t	1.64 s	1.03 d	0.97 s
Dactylol (III)	5.31 t	1.82 s	0.92 d	0.90 s

tracts were combined and the pentane evaporated at reduced pressure. The pentane extract (37 g) was subjected to silica gel column chromatography (E. Merck, 70–230 mesh) using hexane as eluent. All nonpolar fractions were checked by tlc and glc, and fractions containing I were combined. Further purification using preparative glc (15% Carbowax on Chrom P, 140°C, retention time: 14 min) yielded 30 mg of oil. To avoid complete decomposition, the material was kept as a soln in pentane and was stored in a freezer. Retention time on analytical columns: 12% OV-17 on Gas Chrom Q, 155°C, 7.5 min, 3% OV-25 on Gas Chrom Q, 110°, 5.9 min; IR (neat) 899 $\mathrm{cm^{-1}}$, NMR ($\mathrm{CDCl_3}$) 0.97 (gem-diMe, s, 6H), 1.03 ($-\mathrm{CH}-\mathrm{C\underline{H}_3}$, d, J = 5.2, 3H), 1.64 ($\mathrm{C{=}C{-}C\underline{H}_3}$, s, 3H), 2.4 (m, 2H), 2.91 (q, J = 11.5, 1H), 3.54 (m, 1H), 5.06 ($\mathrm{C{=}OH}$, s, 1H), 5.34 ($\mathrm{C{=}CH}$, t, J = 8.4, 1H); MS: *m/e* 204 ($\mathrm{M^+}$, 6%), 189 (5), 150 (10), 137 (11), 136 (100), 135 (18), 121 (58), 107 (19), 93 (22), 91 (14), 79 (12), 55 (11), 41 (19).

Dehydration of dactylol (III). Dactylol[9] (80 mg) was dissolved in 4 ml pyridine, $\mathrm{POCl_3}$ (1.2 ml) was added dropwise at 0° and the mixture was allowed to remain at room temp. for 4 days. The darkened soln was poured cautiously into a mixture of ice and pentane, the aqueous and organic layers were separated and the former extracted with pentane. The pentane extracts were combined, washed with cold 10% HCl, cold 10% $\mathrm{Na_2CO_3}$, satd. NaCl soln and water respectively, and dried over $\mathrm{Na_2SO_4}$. Glc analysis of the mixture showed a peak (with a shoulder) of retention time 5.8 min (3% OV-25 on Gas Chrom Q, 110°); UV λ 232 nm; MS: $\mathrm{M^+}$ 204 for both the shoulder and the peak.

Hydrogenation of dactylol dehydration products and precapnelladiene. The dactylol dehydration mixture was dissolved in MeOH without further purification and hydrogenated at room temp. under normal pressure using Pd/C (10%) as catalyst for 48 hr. The catalyst was removed by filtration and the stereoisomeric mixture subjected to hplc fractionation (pressure: 550 psi, flow rate: 2.9 ml/min, solvent, abs MeOH). The mixture gave a broad peak with a shoulder, retention time: 49.5 min. Five fractions from the same peak were collected. To avoid partial loss of stereoisomers because of their slight volatility during evaporation of MeOH, pentane was added to each fraction. After the addition of small amounts of water, the pentane layers were separated and the methanol–water layers were extracted with pentane. The combined pentane solns were evaporated under reduced pressure at 20°. Every hplc fraction was checked by glc. The third fraction, which contained the major stereoisomer, was subjected to preparative glc for final purification using a column of 12% OV-17 at 160°; retention time: 22 min. The same stereoisomer from precapnelladiene was obtained following the above mentioned technique. Retention time on analytical column (3% OV-25 on Gas Chrom Q, 110°): 5.2 min; NMR (360 MHz) 0.87 (gem-diMe, s, 6H), 0.90–0.94 (secondary Me's, two doublets, 6H); MS: *m/e* 208 ($\mathrm{M^+}$, 3%), 193 (13), 152 (17), 137 (25), 123 (16), 97 (34), 96 (51), 95 (86), 83 (29), 81 (93), 69 (57), 67 (46), 55 (100).

Acknowledgements—We are grateful to Prof. Bernard Tursch (University of Brussells) for collecting the specimens of *Capnella imbricata*. We also wish to express our appreciation to Dr. W. Fenical, Institute of Marine Resources, Scripps Institute of Oceanography, La Jolla, for helpful suggestions and a generous donation of dactylol. Financial support from the National Institutes of Health (Grants No. GM 06840 and RR 00612) and the Stanford University Medical Experimental Medical Facility, SUMEX (RR-785) is gratefully acknowledged. NMR spectra were taken under the supervision of Dr. Lois J. Durham. Grants from the National Science Foundation for the use of a 100 MHz NMR spectrometer (Grant GP 29142) equipped with a computer (Grant CHE-77-08810) as well as grants from the National Institutes of Health (RR 00711) and from the National Science Foundation (GP 23633) for use of a 360 MHz spectrometer are also acknowledged.

REFERENCES

[1] For paper LXXV, see E. Ayanoglu, T. Gebreyesus, C. M. Beechan, C. Djerassi and M. Kaisin, *Tetrahedron Letters* 1671 (1978).

[2] R. E. Carhart, D. H. Smith, H. Brown and C. Djerassi, *J. Am. Chem. Soc.* **97**, 5755 (1975).

[3] C. J. Cheer, D. H. Smith, C. Djerassi, B. Tursch, J. C. Braekman and D. Daloze, *Tetrahedron* **32**, 1807 (1976).

[4] D. H. Smith and R. E. Carhart, *Ibid.* **32**, 2513 (1976).

[5] M. Kaisin, Y. M. Sheikh, L. J. Durham, C. Djerassi, B. Tursch, D. Daloze, J. C. Braekman, D. Losman and R. Karlsson, *Tetrahedron Letters* 2239 (1974).

[6] Y. M. Sheikh, G. Singy, M. Kaisin, H. Eggert, C. Djerassi, B. Tursch, D. Daloze and J. C. Braekman, *Tetrahedron* **32**, 1171 (1976).

[7] Y. M. Sheikh, C. Djerassi, J. C. Braekman, D. Daloze, M. Kaisin, B. Tursch and R. Karlsson, *Ibid.* **33**, 2115 (1977).

[8] R. Karlsson, *Acta Crystallogr. Sect. B* **32**, 2609 (1976).

[9a] F. J. Schmitz, D. C. Campbell, K. Hollenbeak, D. J. Vanderah, L. S. Ciereszko, P. Steudler, J. D. Ekstrand, D. Van der Helm, P. Kaul and S. Kulkarni, In *Marine Products Chemistry* (Edited by D. J. Faulkner and W. H. Fenical), p. 293. Plenum, New York (1977); [b] F. J. Schmitz, K. H. Hollenbeack and D. J. Vanderah, *Tetrahedron* in press.

[10] K. Ziegler and H. Wilms, *Ann.* **567**, 1 (1950).