Tetrahedron. Vol. 30, pp. 3615 to 3620. Pergamon Press 1974. Printed in Great Britain

SOME NEW CEMBRANE DERIVATIVES OF MARINE ORIGIN

Y. KASHMAN*, E. ZADOCK and I. NÉEMAN
Department of Chemistry, Tel-Aviv University, Tel-Aviv, Israel

(*Received in the UK 23 May* 1974; *Accepted for publication 7 June* 1974)

Abstract—The isolation and structural determination of several cembrane derivatives (2-7) isolated from the soft-bodied coral *sarcophytum glaucum* - is reported. The various compounds can be divided into cembranolids, cembranes containing the dihydrofurane moiety, and correlated alcohols. The stereochemistry at C-1, as well as that of the epoxide ring, believed to be responsible for the differences between the members of the first two classes of compounds, is discussed.

In a previous publication[1] we described the isolation and structural elucidation of a new cembranolide, namely sarcophine (**1**), isolated from the soft bodied coral, *sacophytum glaucum* (Alcyonaria). Herewith several other closely related compounds are described. We found that in addition to **1** which crystallizes out from the petrol-ether extract, this extract contains several other diterpenes, some of which (**2** and **3**) appear in considerable amounts (up to 4·5% dry weight). Moreover, the relative amounts of the various diterpenes in diverse batches of *sarcophytum glaucum* changed remarkably as a function of the collection location and period of the year, e.g. the amounts of **1** changed from ca. 3%, dry weight, to traces.

Compounds **2** and **3** were found to be very similar in almost all the chemical and spectroscopic properties (bp., TLC, IR, and Mass spectra); the differentiation could only be based on very slight differences observed in the chemical shifts, in the NMR spectra.

Compound **2**, $C_{20}H_{30}O_2$ (*m/e* 302; M^+), $bp_{0.01}$ 120°, was suspected to possess two ethereal linkages, as neither OH nor CO absorption could be observed in the IR spectrum. Fig 1 illustrates the NMR spectrum and the structure of **2**. The latter was deduced from the spectrum shown and by the comparison of it with that of sarcophine (**1**)[1]. Three out of the four Me-groups appear at δ-values (1·19, 1·57 and 1·76) similar to the ones found for sarcophine, suggesting a priori similar functional groups, namely two three-substituted double bonds and one oxirane. The existence of an epoxide was further confirmed by the triplet at δ 2·53 (J = 4·5 Hz) which is in a suitable place for an oxirane methine.[2] Furthermore, one of the three-substituted double bonds was established by the triplet at 5·05 (J = 6·5 Hz) *vide infra*. The picture at low field turned out to be more complicated; two protons appear as a broad doublet at δ 4·34 while three additional ones gave rise to the multiplet between 5·0 and 5·5 ppm. Of most significance in this low-field pattern elucidation, was the double resonance experiment in which the two Me-groups at 1·57 and 1·61 ppm were irradiated simultaneously. This spin decoupling cancelled long range couplings caused by these two methyls (Fig 1, irrad at δ 1·60); a sharp doublet appears at δ 4·34 (J = 4·5 Hz, 2H), and the multiplet between 5·0 and 5·5 ppm could be interpreted as an AB-system (J_{AB} = 9Hz, 2H) overlapping a triplet at 5·05 (J = 6·5 Hz, 1H). The AB proton appearing at lower field (δ 5·37) was found to be further splitted into a triplet (J = 4·5 Hz) by two protons suspected to be the ones giving rise to the doublet at 4·34 (J = 4·5 Hz), and indeed irradiation at this place changed the 5·37-signal into a broad doublet (Fig 1, irrad at δ 4·34). Vice versa irradiation at δ 5·37 changed the broad doublet at 4·34 into a broad singlet (Fig 1), as well as causing sharpening of the methyl signals at δ 1·61 and 1·76, as compared to the 1·57 signal. The AB-pattern resembles the one appearing in the NMR spectrum of **1** except for the additional splitting of one proton which is caused by two neighbouring protons. The absence of the γ-lactone appearing in **1**, together with the need for an ethereal linkage, in addition to the epoxide, suggests the existence of a 2,5-dihydrofurane site in **2**, in place of the γ-lactone of **1**. A dihydrofurane moiety can account for the signal at δ 5·37, representing the proton α to the oxygen coupled by the two other α'-protons, to an extent of 4·5 Hz which is characteristic for such systems.[3] Furthermore, the position of the various protons including the methyl appearing at δ 1·61 is thus also explained.[4] The other half of the AB-pattern, appearing at δ 5·12, is therefore attributed to C-2-H and is allylicly coupled with the Me-group appearing at δ 1·76. Irradiation of the latter Me-group

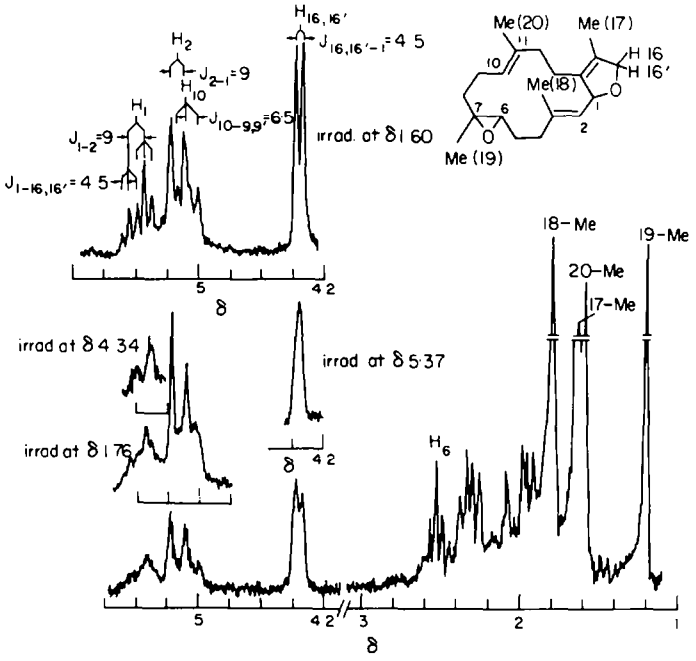
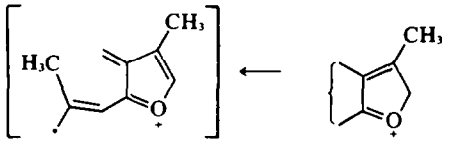


Fig 1. NMR Spectrum of compound 2.

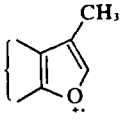
sharpened the doublet at δ 5·12, while the beginning of five lines could be observed on the multiplet at δ 5·37, due to cancelling of the homoallylic coupling (Fig 1).

More evidence for the existence of the dihydrofurane moiety could be obtained from the mass spectrum in which the M-1 and M-2 fragments appear in relatively high abundance, explained by structure **a** and **b**.



c 148 (100%)

a 301 (13%)



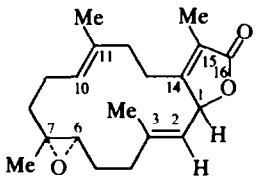
b 300 (60%)

Fragment **a** can also account for the parent peak, at *m/e* 148, for which structure **c** is proposed. The problem with the mass spectrum of **2** (or **3**), as discussed before for compound **1**,[1] was the large number of possible fragmentations due to the various functional groups. Thus further conclusions concerning the relative positions of the functional sites became very difficult; several mechanisms could be suggested for most of the peaks. On the ground of the above data, mainly the NMR spectrum, and the assumption of a similar biosynthesis (including the place of epoxidation) for **2** and **1**, the proposed structure for **2** is the one shown in Scheme 1.

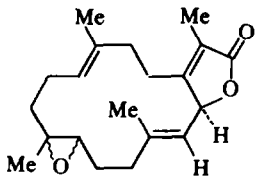
As mentioned before, compound **2** was found to be accompanied by a very similar diterpene **3**, which could be separated from it by repetitive chromatography. According to the NMR data of **3** as compared to **2** (see Table 1) compound **3** contains exactly the same functional groups as **2**.

If we assume that neither double bond rearrangement nor *cis-trans* isomerisation occurs (on the basis of the very similar spectral data and the common cembrane derivative structures)[5] the only remaining differentiation between the two (**2** and **3**) could originate from the stereochemistry of one or more of the C-1, C-6 or C-7 centers. We have no way of making an unequivocal decision among these possibilities, however, as the differences between **2** and **3**, seen in the NMR spectrum (Table 1), are mainly in the dihydrofurane surrounding, we assume C-1 epimerization; further evidence will be discussed later.

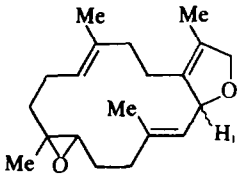
On careful chromatography of the crude oily petrol-ether extract, using benzene-ethylacetate as the eluent, another diterpene (**4**), apart from compounds **2** and **3**, could be obtained. Compound **4**, $C_{20}H_{28}O_3$ (*m/e* 316; M^+), m.p. 70°, was found to possess an IR and mass spectrum undistinguishable from that of **1**, however very slight differences



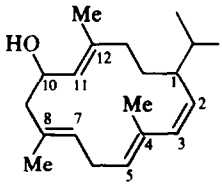
1



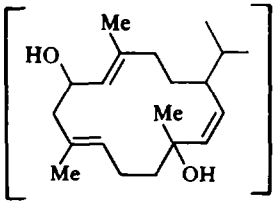
4,5 (differ in the epoxide configuration)



2,3 (differ in H_1-configuration)



6



7 (tentative structure)

SCHEME 1

Table 1. NMR data of compounds 2 and 3†

Compound / proton	2	3
(17) CH_3	1·61 brs*	1·65 brs*
H_{16}	4·34 brd (J = 4·5 Hz)	4·34 brs*
$H_{16'}$		
H_1	5·37 dt* (J = 9; 4·5 Hz)	5·10–5·25m (2H)
H_2	5·12d (J = 9 Hz)	
CH_3(18)	1·76 brs*	1·85 brs*
(20) CH_3	1·57 brs*	1·57 brs*
H	5·05 brt* (J = 6·5 Hz)	5·05 brt* (J = 6·5 Hz)
(19) CH_3	1·18 s	1·19 s
	2·53 t (J = 4·5 Hz)	2·51 t (J = 5 Hz)

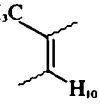
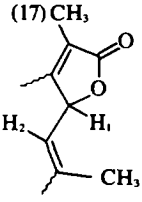
*Further splitted by allylic couplings.
†Taken in CCl_4.

could be observed in the NMR spectrum (Table 2). Double irradiation experiment showed exactly the same behaviour as 1,[1] suggesting, together with the other spectral data, that 4 is a stereoisomer of 1. As such, compound 4 was expected to exhibit a cotton effect (in contrast to compounds 2 and 3), and indeed, the measured CD-curve showed this effect (Fig 2). Moreover, this effect differed significantly from the one found for 1, i.e. the cotton effect attributed to the $n \rightarrow \pi^*$ lactone absorption changed in sign. Dealing with two unequal partly overlapping cotton effects ($n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$) the place of both maxima is expected to be strongly dependant on the intensity of the separate absorptions,[6] thus explaining the shift in the maxima observed. However, on the basis of the CD-interpretation of compound 1, it seems most likely that the C-1 configuration of 4 is inverted, as compared to 1, and that this is the structural feature which distinguishes between the two.

Another interesting observation was the fact that if the chromatography of the crude extract was carried out, using $CHCl_3$ instead of EtOAc, compound 4 was absent and instead, another compound (5) appeared.

Compound 5, an oil, $C_{20}H_{28}O_3$ (*m/e* 316; M^+) showing undistinguishable TLC, IR and mass spectrum from those of 1 and 4, but slight differences in the NMR spectrum (Table 2), seemed to point to yet another stereoisomer. The CD spectrum of 5 shows a positive $n \rightarrow \pi^*$ cotton effect, similar to that of 4, pointing to the same C-1 configuration. We assume that in compound 5 epimerization of one of the carbons bearing the epoxide moiety occurs. It was very interesting to find that compound 4 changes into 5 while the NMR spectrum is being run in $CDCl_3$ solution (but not in CCl_4) and turns back to 4 when the $CDCl_3$ is evaporated and the spectra taken this time in CCl_4. The explanation for this unexpected behavior can be sought in the following two factors: (1) the acidity of the $CDCl_3$ and/or (2) the traces of phosgene present in the $CDCl_3$. It actually turned out that traces of $COCl_2$ were responsible for the isomerization in $CDCl_3$ and most likely also on the chromatography column, using chloroform as eluent. Adding traces of phosgene to an NMR tube containing 4 in CCl_4 caused duplicity of the

Table 2. NMR data of compounds 1, 4 and 5*

Compound / proton	1	4	5
(19) H_3C 	1·28 s 2·68 t (J = 5)	1·21 s 2·50 t (J = 5)	1·26 s 2·66 t (J = 5·5)
(20) H_3C 	1·63 brs 5·15 t (J = 6)	1·61 brs 5·09 t (J = 6)	1·61 brs 5·11 t (J = 6)
(17) CH_3 	1·85 t (J = 1·2) 5·55 d (J = 10) 5·05 d (J = 10) 1·89 d (J = 1)	1·79 t (J = 1·5) 5·34 d (J = 9·5) 4·94 d (J = 9·5) 1·93 d (J = 1)	1·85 t (J = 1·5) 5·43 d (J = 9·5) 4·98 d (J = 9·5) 1·92 d (J = 1)

*Taken in CCl_4 (δ values).

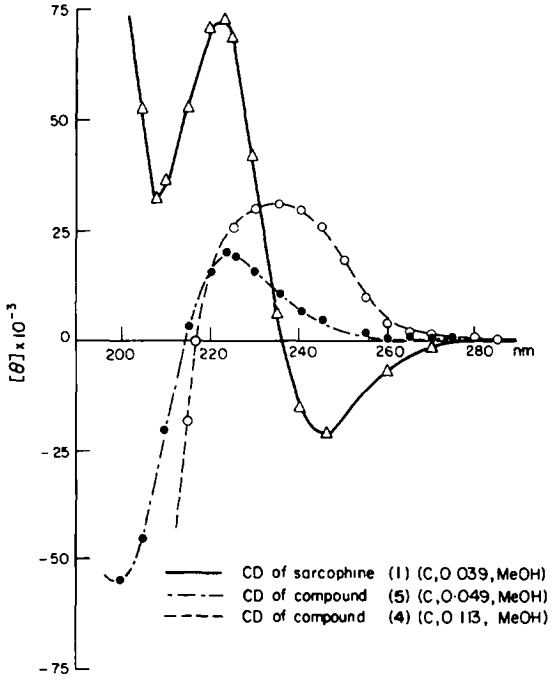
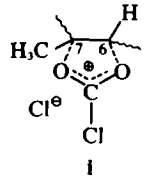


Fig 2. CD of compounds 1, 4 and 5.

Me-signals, showing transformation of 4 into 5. (In the presence of more than traces of phosgene, the epoxide rearranges into a ketone). A possible mechanism may include the opening of the oxirane ring after initial $COCl_2$ attack on its oxygen, and reclosing from the other side, the intermediate being an ion of type i which may be stabilized by the spatial neighbouring double bonds. It is



i

worthwhile to mention that 1 does not show a similar rearrangement pointing out the very delicate situation in 4. The above isomerization makes it doubtful as to whether 5 is a natural product or an artifact.

The observation that in the case of 1, C-1 epimers exist in the coral, affirms the assumption that 2 and 3 are also C-1 epimers, *vide supra*. Full proof can, of course, be obtained only by degradation, which, had it been performed, because of the great lability of the compounds, could not have given us unequivocal results.

Among the diterpenes which appear as minorities in the crude extract, we succeeded in the isolation of small amounts of two polar compounds (6 and 7).

Compound 6, $C_{20}H_{32}O$, (*m/e* 288; M^+) m.p. 143°–145°, ν_{max}^{KBr} 3200 (OH), 1670, 980, 965 cm^{-1} (C=C) had the following NMR spectrum: 0·81 d (J = 6Hz, 3H) and 0·86 d (J = 6Hz, 3H) an isopropyl group, 1·60 d (J = 1·5 Hz, 3H), 1·68 d (J = 1Hz, 3H) and 1·77 t (J = 1·5 Hz, 3H)—three vinyl methyls, 5·97 d (J = 15 Hz, 1H), 5·50 t (J = 7 Hz, 1H) and

5·0 – 5·2 m (3H)—5 vinyl-protons and 4·51 ddd (J = 11; 8 and 5 Hz, 1H)—a proton α- to OH group. The existence of 3 vinyl Me groups and 5 vinyl protons (four unsaturations out of the molecules' five) together with the presence of an iPr-group indicate the existence of a cembrene skeleton (containing four double bonds in the macrocycle). The Me at δ 1·77 together with the doublet at 5·97 point to a diene, like the one found in cembrene and another cembranolide.[5a-c]

If we assume the cembrene skeleton, the only detail which needs clarification is the location of the OH. The signal at δ 4·51 is in agreement with a –CHOH group in an allylic position,[5d,7] which leaves us with four possible places (carbons 6, 9, 10 and 13). According to the multiplicity of the CHOH proton, three vicinal protons are requested, which takes out of consideration carbons 6, 9 and 13, leaving C-10 as the only place. The coupling constants found for this proton agree with the expected values according to the literature.[5d,7b,8] Structure **6** as shown in Scheme 1 is the proposed one; the small amounts of **6**, which did not appear in every examined batch of the coral, made further confirmation impossible.

Compound **7**, $C_{20}H_{34}O_2$ a diol, (*m/e* 306; M^+) ν_{max}^{KBr} 3350 (OH), 1670, 965 cm^{-1} (C=C) appeared in even smaller amounts than **6** and actually only traces were obtained. Compound **7** showed the following NMR spectrum: δ 0·82 d (J = 7 Hz, 6H)—an isopropyl group, 1·34 s (3H)—$CH(OH)CH_3$, 1·63 brs (3H) and 1·65 brs (3H)—two vinyl methyls, 5·50 d (J = 15 Hz, 1H) and 5·0–5·3 m (3H)—4 vinyl protons, and 4·45 ddd (J = 11; 8 and 5 Hz, 1H)—CH(OH).

It seems to us that in **7** the conjugated diene (of **6**) at C_2-C_5 is replaced by one disubstituted double bond (C_2-C_3) and one tertiary hydroxyl group on C_4, as shown tentatively in Scheme 1. Another possible structure in which the second hydroxyl is located at C_6 is known in the literature[7a] but is not in agreement with our diol.

We intend to further investigate the distribution of these diterpenes in *sarcophytum glaucum* and other closely related corals.

EXPERIMENTAL

M.ps were taken on a Thoms Hoover capillary m.p. apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Infracord model 337 spectrophotomer. NMR were taken on a Varian HA-100 spectrometer using 5–10% soln. in $CDCl_3$, with TMS as an internal standard. Mass spectra were taken with an Hitachi Perkin-Elmer RMU 6 instrument. CD spectra were taken on a Cary 60 recording spectropolarimeter with the Model 6006 CD attachment. α_D were taken on a Pepol 60 spectropolarimeter.

Isolation procedure of compound **5**. The fresh soft coral material (300 g, dry weight) was extracted during 24 h with petrol-ether in a soxhlet. After filtration of **1** which crystallized out on cooling (6 g), the petrol-ether extract was evaporated and the oily residue obtained (*ca* 35 g) chromatographed on a florisil column (60–100 mesh). Repetitive chromatographs of the fractions eluted with petrol-ether: $CHCl_3$ (1:1) (5 g) gave **5** (0·52 g). Compound **5**, a colourless oil, could not be allowed to crystallize. Its mass spectrum was identical with that of **1**, the CD and NMR data are given in Fig 2 and Table 2 respectively. ν_{max}^{neat} 2920, 2850, 1750, 1675, 1450, 1390, 1320, 1300, 1235, 1090, 1070, 995, 860, 775 cm^{-1}.

Isolation of compounds **2** *and* **4**. A crude petrol-ether extract of *sarcophitum glaucum* (8 g) was chromatographed on a silica gel column (0·05–0·20 mm, Merck 250 g). Elution with benzene-EtOAc (3:1) gave **2** (1·07 g) while further elution with benzene-EtOAc (1:1) gave **4** (150 mg). Compound **4**, m.p. 70° (CCl_4), $[\alpha]_D^{25°} = -16°$ (*c*, 1·19, MeOH), showed the same mass spectrum as **1** and **5**. The CD and NMR data are given in Fig 2 and Table 2 respectively; ν_{max}^{KBr} 2950, 2890, 1750, 1675, 1450, 1390, 1330, 1310, 1235, 1095, 1070, 995, 870, 850, 790, 765 cm^{-1}.

Compound **2** is a colourless oil, $b.p._{0.05}$ 120°, (Found: C, 79·60; H, 9·81: $C_{20}H_{30}O_2$ requires: C, 79·42; H, 10·00%). $[\alpha]_D^{25°} = +40°$ (*c*, 2·20, MeOH), ν_{max}^{neat} 2900, 2830, 1675, 1450, 1380, 1230, 1215, 1040, 945, 770 cm^{-1}. Mass spectrum (*m/e*, %): 302 (25, M^+), 301 (13), 300 (60), 284 (26), 283 (13), 282 (60), 175 (27), 163 (55), 150 (40), 148 (100), 135 (60), 109 (45), 95 (30), 84 (75), 71 (75). The NMR spectrum is given in Table 1.

Isolation of compound **3**. The crude petrol-ether extract (10 g) was chromatographed on silica gel (300 g). The fraction eluted with benzene-EtOAc (3:1), (2 g) contained mainly **3**, 300 mg of this fraction were rechromatographed on a silica-gel-H column (60 g, 1·5 cm I.D., 100 cm length) to give pure **3** (200 mg). Compound **3**, an oil $b.p._{0.05}$ 120°, IR and mass spectrum identical with that of **2**. The NMR data are given in Table 1.

Isolation of compounds **6** *and* **7**. Rechromatography of the polar fraction (1 g), obtained from the crude extract (30 g), on silica gel (70 g) gave by elution with petrol-ether: $CHCl_3$ (1:2) **6** (25 mg), m.p. 143°–145° (petrol-ether; acetone), Found: *m/e* 288 M^-, $C_{20}H_{32}O$; requires: 288. ν_{max}^{KBr} 3200, 2950, 2920, 2860, 1670, 1450, 1435, 1380, 1260, 1110, 1030, 1010, 980, 865, 840 cm^{-1} Mass spectrum (*m/e*, %): 288 (65, M^-), 273 (12), 270 (20), 255 (10), 245 (20), 227 (30), 204 (25), 190 (45), 161 (65), 149 (50), 135 (85), 107 (75), 93 (90), 69 (55), 43 (100); NMR: see text.

Compound **7**, was obtained in *ca* 90% purity only. The small amount (20 mg) prevented crystallization. ν_{max}^{neat} 3350 (strong), 2900, 1660, 1450, 1380, 1100, 1040, 970, 930, 860, 800, 750 cm^{-1}; NMR: see text.

REFERENCES

[1a] J. Bernstein, U. Shmueli, E. Zadock, Y. Kashman and I. Néeman, *Tetrahedron* in press; [b] I. Neeman, L. Fishelson and Y. Kashman, *Toxicon* in press

[2a] J. M. Jackman and S. Sternhell, *Applications of NMR Spectroscopy in Organic Chemistry* p. 227. Pergamon Press Oxford (1969); [b] R. T. Aplin and L. Coles, *Chem. Commu.* 858 (1967)

[3a] L. F. Johnson, A. V. Robertson, W. R. J. Simpson and B. Witkop, *Aust. J. of Chem.* **19**, 115 (1966); [b] C. C. J. Culvenor, M. L. Heffernan and W. G. Woods, *Ibid.*, **18**, 1607 (1966); [c] K. Takeda, K. Tori, I. Horibe, M. Ohtouru and H. Minato, *J. Chem. Soc.* (C), 2697 (1970)

[4a] S. Sternehll, *Rev. Pure App. Chem.* **14**, 15 (1964); [b] See Ref. 3*c*; [c] J. P. Gouesnard and G. J. Martin, *Bull. Soc. Chim. Fr.* 4452 (1969); [d] E. E. Schweizer and J. G. Liehr, *J. Org. Chem.* **33**, 583 (1968)

[5a] W. G. Dauben, W. E. Thiessen and P. R. Resnick, *Ibid.*

30, 1693 (1965); [b] M. G. B. Drew, D. H. Templeton and A. Zalkin, *Acta Cryst.* **B25**, 261 (1969); [c] B. Kimland and T. Norin, *Acta Chem. Scand.* **22**, 943 (1968); [d] V. D. Patil, U. R. Nayak and S. Dev, *Tetrahedron* **29**, 341 (1973)
[6] K. M. Wellman, P. H. A. Laur, W. S. Briggs, A. Moscowitz and C. Djerassi, *J. Am. Chem. Soc.* **87**, 66 (1965)
[7a] D. L. Roberts and R. L. Rowland, *J. Org. Chem.* **27**, 3989 (1962); [b] E. L. Ghisalberti, P. R. Jefferies, T. G. Payne and G. K. Worth, *Tetrahedron* **29**, 403 (1973)
[8] A. J. Weinheimer, R. E. Middlebrook, J. O. Bledsoe, Jr., W. E. Monsico and T. K. B. Karns, *Chem. Commu.* 384 (1968)