Tetrahedron Vol. 34, pp. 1551–1556
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0040-4020/78/0501-1551/$02.00/0

CHEMICAL STUDIES OF MARINE INVERTEBRATES—XXVIII[1,2]

DITERPENES FROM *CLAVULARIA INFLATA* (COELENTERATA, OCTOCORALLIA, STOLONIFERA)

J. C. BRAEKMAN,† D. DALOZE,* R. SCHUBERT, M. ALBERICCI and B. TURSCH
Collectif de Bio-écologie, Faculté des Sciences, Université Libre de Bruxelles, Belgium

and

R. KARLSSON
Structural Chemistry, Arrhenius Laboratory, University of Stockholm, Sweden

(*Received in UK 21 February 1977; Accepted for publication 22 April 1977*)

Abstract—The presence of terpenoids in the order Stolonifera has been established by the isolation of three novel diterpenes, 1α,4β-dihydroxyclavular-17-ene (1), 4β-hydroxyclavulara-1(15),17-diene (2) and 3α,4β-dihydroxyclavulara-1(15),17-diene (3) from *Clavularia inflata*. The structure of 1 has been determined by X-ray diffraction analysis and those of 2 and 3 by chemical intercorrelation with 1.

Octocorals have been shown, during the last decade, to be an abundant source of terpenoid compounds.[3] It has to be stressed that until now these chemicals have been reported solely from Gorgonacea and Alcyonacea, two only of the five orders that constitute the subclass Octocorallia.

During a preliminary survey of the marine invertebrates of Laing Island (Papua-New Guinea), great concentrations of the Stolonifer *Clavularia inflata* Schenk, 1896, were noticed at depths between 15 and 35 m, on the outer reef slopes of the island. This provided us with the long awaited opportunity to check the presence of terpenoids in the order Stolonifera, that, like the Gorgonacea and the Alcyonacea, does also contain symbiotic zooxanthellae.[4] Moreover the local occurrence of abundant, rather homogeneous populations colonizing dead hermatypic coral surfaces suggested the presence of allomones.

The dichloromethane extract of sun-dried *Clavularia inflata* was submitted to repetitive silica gel column chromatography, yielding three compounds, 1, 2 and 3, amounting to more than 1% of the dry weight of the animal.

The NMR spectrum of compound 1 ($C_{20}H_{34}O_2$) shows four singlets of 3H each at 0.90, 0.94, 1.14 and 1.67 ppm, attributed respectively to two tertiary Me groups, one Me group vicinal to an O atom and one vinyl Me. A ν_{OH} absorption at 3400 cm^{-1} in the IR spectrum of 1, in conjunction with a multiplet of 1H at 3.20 ppm (shifted to 4.47 ppm in the spectrum of the monoacetate 4) indicates the presence of a secondary OH function, further proved by oxidation of 1 into the ketone 5 ($\nu_{C=O}$ at 1695 cm^{-1}). The IR spectra of the monoacetate 4 and the ketone 5 still present a ν_{OH} absorption band, establishing that the second O atom of 1 belongs to a tertiary alcohol. Other spectral characteristics of 1 (2H broad singlet at 4.68 ppm and IR bands at 1640 and 880 cm^{-1}), indicate the existence of a $C{=}CH_2$. With no evidence for the presence of further double bonds, it could thus be concluded that 1 is tricyclic and that it possesses an isopropenyl group. Since these structural features could not be incorporated in any diterpene skeleton known at the time‡ compound 1 was submitted to single crystal X-ray diffraction analysis.

Crystals of compound 1 belong to the space group $P2_12_12_1$ with $a = 15.66$, $b = 16.92$, $c = 14.10$ Å and two independent molecules in the unit cell. Independent X-ray intensities with $\theta < 70°$ were collected with CuK_α radiation on a diffractometer. The structure was solved by direct methods and refined with isotropic thermal parameters (Table 1) by full-matrix least-squares to the conventional residual $R = 0.105$ using the 1863 most significant intensities. The H atoms were included in the refinement with fixed isotropic parameters. The H positions were calculated from geometrical considerations with C–H 1.0 Å.

Figure 1 gives the relative conformations of the two molecules denoted A and B. The OH groups are involved in four H-bonds: O(1)A–H...O(2)B (2.66 Å), O(2)A–H...O(1)B (2.73 Å), O(1)B–H...O(1)A (2.86 Å), and O(2)B–H...O(2)A (2.75 Å).

The work described here does not define the absolute configuration of 1.

Structure 1 is closely related to that of dolatriol 6, a cytotoxic diterpene recently isolated from the mollusk *Dolabella auricularia*,[5] for whose skeleton the name dolastane has been coined. It is unfortunate that the new skeleton dolastane (20) was defined with stereochemical implications at C-1 and C-9 that are not necessarily linked to the stereochemistry of the natural parent hydrocarbon, since several asymmetric carbons are functionalized.

Furthermore it does not define the *cis* or *trans* nature of the ring junctions. In order to obviate these shortcomings we feel thus compelled to recommend the new name clavularane for the skeleton depicted in 21 with the substituents designated β if they are orientated on the side of the C-5 Me group and α in the opposite case: Compound 1 thus becomes 1α,4β-dihydroxyclavular-17-ene.

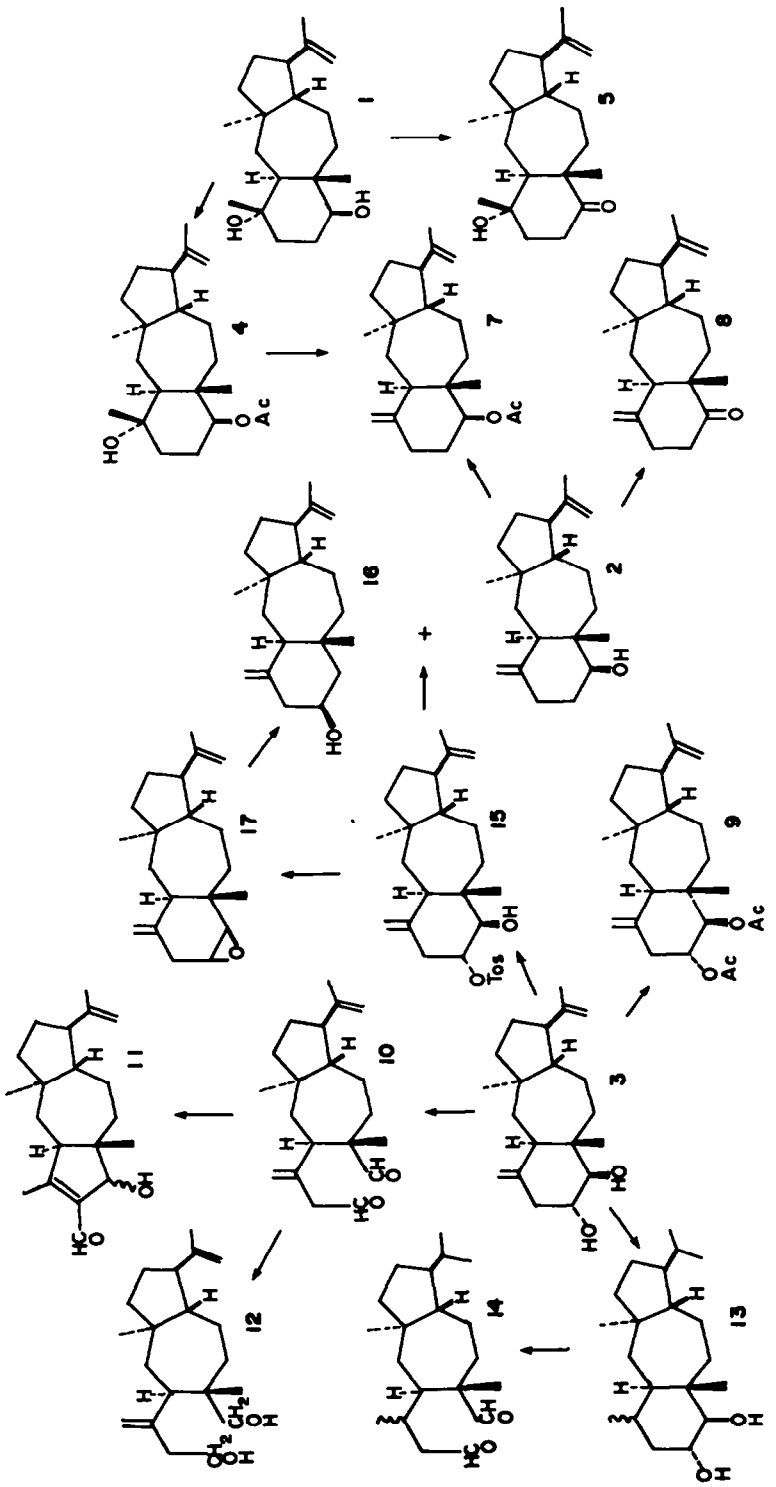
†Chercheur qualifié du Fonds National de la Recherche Scientifique belge.

‡The structure of dolatriol (6)[5] was published after completion of this work.

Table 1. Atomic coordinates ($\times 10^4$) and thermal parameters ($\times 10$ Å^2) with e.s.d.'s in parentheses

Molecule A	x	y	z	B
C(1)	-1060(9)	2057(8)	634(10)	29(3)
C(2)	-1050(11)	2829(10)	81(12)	37(4)
C(3)	-355(10)	3391(9)	448(11)	29(3)
C(4)	535(9)	2980(8)	307(10)	24(3)
C(5)	621(9)	2186(8)	844(10)	24(3)
C(6)	1438(9)	1823(9)	395(10)	29(3)
C(7)	1931(10)	1142(10)	892(12)	38(4)
C(8)	1359(11)	500(10)	1438(12)	39(4)
C(9)	1891(11)	-284(11)	1523(13)	44(4)
C(10)	1199(13)	-956(12)	1892(15)	59(5)
C(11)	309(11)	-520(11)	1583(13)	47(4)
C(12)	526(10)	231(9)	933(11)	34(3)
C(13)	-210(9)	825(8)	1102(10)	25(3)
C(14)	-166(9)	1635(8)	580(10)	25(3)
C(15)	-1399(11)	2185(10)	1872(12)	38(4)
C(16)	834(12)	-8(11)	-148(13)	45(4)
C(17)	2541(12)	-230(11)	2335(13)	47(4)
C(18)	2243(16)	-188(15)	3328(18)	78(7)
C(19)	3408(14)	-155(13)	2109(15)	79(5)
C(20)	730(10)	2331(9)	1941(11)	28(3)
O(1)	1189(6)	3517(6)	848(7)	32(2)
O(2)	-1674(6)	1524(6)	158(7)	40(2)

Molecule B	x	y	z	B
C(1)	3248(11)	5148(10)	3550(13)	36(4)
C(2)	2392(12)	5087(11)	2950(13)	45(4)
C(3)	2507(10)	4548(9)	2083(11)	36(3)
C(4)	2728(9)	3716(9)	2428(10)	28(3)
C(5)	3818(9)	3875(8)	2955(10)	25(3)
C(6)	3829(9)	2809(8)	3398(10)	28(3)
C(7)	4478(10)	2474(10)	3751(11)	40(3)
C(8)	5113(10)	3039(9)	4264(11)	33(3)
C(9)	5772(11)	2587(11)	4813(13)	37(4)
C(10)	6200(12)	3268(11)	5511(13)	51(4)
C(11)	5542(12)	3949(11)	5533(14)	47(4)
C(12)	4750(9)	3670(9)	4985(10)	32(3)
C(13)	4384(9)	4385(8)	4405(10)	27(3)
C(14)	3559(8)	4299(8)	3803(10)	26(3)
C(15)	3919(12)	5685(11)	3017(13)	44(4)
C(16)	4085(10)	3326(10)	5683(12)	46(4)
C(17)	6424(10)	2098(9)	4354(11)	39(3)
C(18)	6881(12)	2494(11)	3550(14)	49(4)
C(19)	6571(12)	1344(12)	4808(14)	46(4)
C(20)	4356(9)	3818(9)	2241(10)	30(3)
O(1)	2744(6)	3187(6)	1633(7)	33(2)
O(2)	3018(7)	5530(8)	4428(8)	48(2)



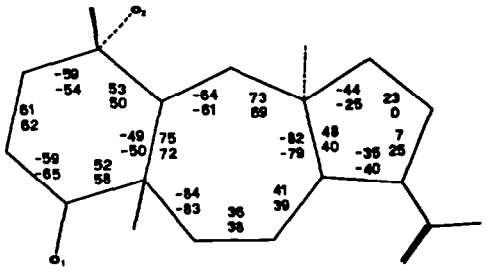


Fig. 1. Relative conformation angles of compound 1 (the bottom values concern molecule B).

The NMR spectrum of compound 2 ($C_{20}H_{32}O$, ν_{OH} 3360 cm^{-1}, $\nu_{C=C}$ 1645 cm^{-1}, $\delta_{=CH_2}$ 885 cm^{-1}) shows the presence of two tertiary Me groups, one vinyl Me and four vinylidene double bond protons. A double doublet at 3.28 ppm (shifted to 4.7 ppm in the spectrum of the monoacetate 7) indicates the presence of a secondary alcohol. Oxidation of 2 affords the unconjugated monoketone 8. Comparison of the spectral properties of compound 2 and 1 and their derivatives, suggests that the methylcarbinol group of 1 is replaced by an exomethylene double bond in 2. This was confirmed by treatment of 4 with $POCl_3$ to yield compound 7. Compound 2 is thus 4β-hydroxyclavulara-1(15),17-diene.

The NMR spectrum of ketone 8 calls for some comment. Indeed, a 4H "singlet" appears at 2.53 ppm, an unexpected feature for such a structure. This was tentatively attributed to an accidental overlap of the four protons at C-2 and C-3, and later confirmed by base-catalyzed deuteriation, leading to a 3,3-dideuterioderivative (M^+ 288), whose NMR spectrum showed a dramatic decrease in the intensity of the peak at 2.53 ppm.

The spectral properties of compound 3 ($C_{20}H_{32}O_2$) suggest a close similarity with compound 2, both tertiary Me groups, the isopropenyl and the exomethylene double bond being still present. A 1H doublet at 2.99 ppm (J 9 Hz) and a 1H multiplet at 3.40 ppm suggest the presence of two secondary alcohols, in agreement with the formation of a diacetate (9).

Fast reaction with $NaIO_4$ indicates the α-glycol nature of these two OH groups. The reaction yields an unstable compound 10 that was not isolated but could clearly be detected by tlc. On attempted purification by silica gel column chromatography, compound 10 was quantitatively rearranged into an hydroxylated monoaldehyde (11), whose structure will be discussed later on.

Direct $NaBH_4$ reduction of the unstable compound 10 affords diol 12 whose NMR spectrum indicates that both OH functions are primary, thus establishing the dialdehyde nature of 10. Furthermore, the $-CH_2OH$ protons of 12 appear respectively as an AB system and a triplet, indicating that one of the hydroxymethyl groups has no neighbouring protons, while the other has two.

Hydrogenation of compound 3 affords a tetrahydro derivative (13), whose treatment with $NaIO_4$ yields a stable dialdehyde (14). Its spectral characteristics (1H s at 9.1 and 1H m at 9.4 ppm) confirm the deductions based on compound 12. On the clavularane skeleton 21, only a 2,3- or a 3,4-diol can meet these requirements. The 2,3-diol solution was considered very unlikely in view of the observed chemical shifts which are not compatible with the presence of an allylic alcohol.

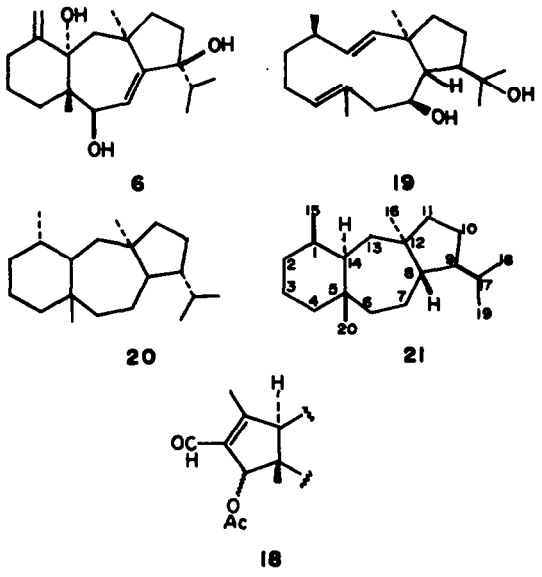
These conclusions were further substantiated by chemical intercorrelation of compounds 2 and 3. Selective tosylation of 3 yields a monotosylate (15) whose NMR spectrum (1H dd at 3.27 and 1H m at 4.40 ppm) indicates that tosylation occurs at position C-3. LAH reduction of 15 in refluxing THF yields two isomeric alcohols, the less polar being identical in all respects (IR, NMR, MS, gc, [α], tlc) with compound 2. This establishes the structure of compound 3 except for the stereochemistry at C-3.

The spectral properties of the more polar alcohol (2H m at 2.35 and 1H m at 4.00 ppm) obtained by reduction of the monotosylate 15 indicate that it possesses structure 16. Formation of this compound is best explained by assuming that the reduction proceeds through the epoxide 17 which can then be further reduced to 2 and 16. Indeed, epoxide 17 could be independently prepared by base treatment of the tosylate 15 and was also shown to be present in the mixture obtained by LAH reduction of compound 15 under milder conditions. Moreover, LAH reduction of the epoxide 17 yielded the same mixture of isomeric alcohols 2 and 16 mentioned hereabove.

Since it has been established that epoxide formation from α-hydroxytosylates implies a *trans* relationship between the reacting groups in the starting product,[6] the OH group at C-3 in 3 must necessarily be equatorial, in full agreement with the observed coupling constants between H-C-4 and H-C-3 (J = 9 Hz).

The H-C-4 proton unexpectedly appears as a double doublet at 3 ppm in the NMR spectrum of compound 15. This has been attributed to an additional coupling between HC-4 and the OH proton, the latter being involved in a strong intramolecular H-bond with the tosylate group. This interpretation was supported by the observation that in the presence of D_2O the double doublet collapsed into the expected doublet (J = 9 Hz).[7]

The structure of the rearranged product 11 can now be discussed in terms of the established structure 3. The labile dialdehyde 10, which is the expected product of metaperiodate cleavage of 3, possesses at C-2 a strongly activated methylene, located between an aldehyde and a double bond. This feature explains the ease of intramolecular cyclization[8] to obtain compound 11 whose spectral characteristics (see experimental) indicate the presence of partial structure 18.[9]



The clavularane skeleton **21** as defined hereabove shows the close relationship existing between compounds **1**, **2**, **3**, dolatriol (**6**) and the dolabellane derivative **19**, recently isolated from opistobranch mollusks.[5,10] Indeed, all the asymmetric centers of **19** have the same relative configuration as their equivalents in the clavularane series and one can easily imagine that the latter could be obtained by a simple cyclization of the dolabellane skeleton. Only one asymmetric center of dolatriol **6** differs from the clavularane series. This could well arise from an isopropylidene equivalent of **1** being hydroxylated from the least hindered side of the molecule.

Clavularia inflata is an interesting example of an Octocoral yielding diterpenes whose type radically departs from the usual cembrane pattern.

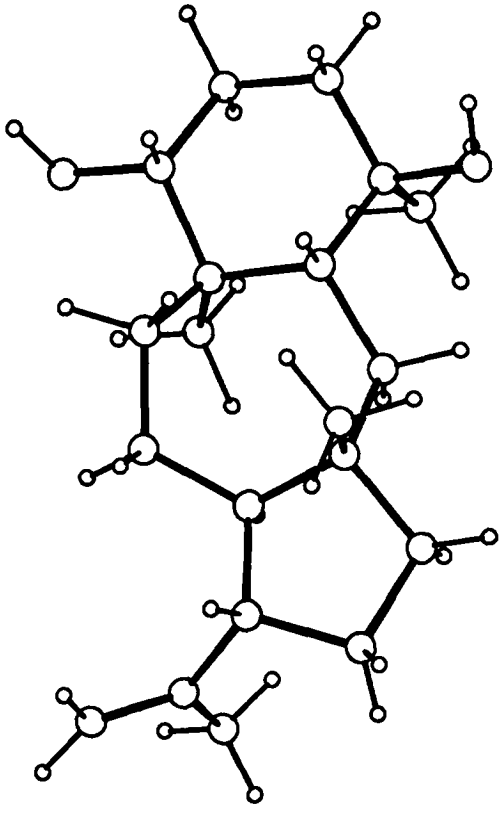


Fig. 2. Computer generated perspective drawing of **1**.

EXPERIMENTAL

The following instruments were used for measuring the physical data. IR: Pye Unicam SP 1000; UV: Pye Unicam SP 800; NMR: Varian T 60, Jeol JNM/MH 100, Perkin–Elmer R24B and Bruker WP 60; Rotation power: Perkin–Elmer 141; MS: Finnigan 3000 D. [α] were recorded at 579 nm (Hg). GLC: Hewlett–Packard 402. Melting points: Arthur Thomas heating stage. High pressure chromatography: Pye LCM 2.

The NMR spectra were recorded in $CDCl_3$ solution. Chemical shifts are quoted in δ values (ppm) downfield from TMS as internal standard. The tlc were performed on Polygram Sil G pre-coated plates (Macherey–Nagel). All described compounds were homogeneous in tlc and GLC.

Isolation of compounds **1**, **2** *and* **3**. Sun-dried specimens of *Clavularia inflata* were finely ground and 977 g of the resulting powder was then extracted in a Soxhlet during 24 hr with CH_2Cl_2. This affords 64.3 g of extract which was chromatographed on a silica gel column (eluted with CH_2Cl_2), to yield three main fractions which were subsequently repurified by repetitive silica gel column chromatography (eluents: dichloromethane, hexane–acetone 8:2 and ether). This procedure yielded **1**, **2** and **3** in 0.08, 0.15 and 0.92% respectively.

1α,4β-Dihydroxyclavular-17-ene (**1**). $C_{20}H_{34}O_2$; m.p. 165–167°; [α] +9° (c = 0.79; $CHCl_3$); IR (film): ν_{OH} 3400 cm^{-1}, δ = CH_2 880 cm^{-1}; NMR: 0.90 and 0.94 (3H s, $^{16}CH_3$ and $^{20}CH_3$), 1.14 (3H s, $^{15}CH_3$), 1.67 (3H bs, $^{19}CH_3$), 3.20 (1H m, ^{4}CH), 4.68 (2H bs, $^{18}CH_2$); MS: 306 (19, M^+), 291 (5, $M^+ - CH_3$), 288 (17, $M^+ - H_2O$), 273 (7, $M^+ - CH_3 - H_2O$), 263 (25, $M^+ - 43$), 250 (22, $M^+ - 56$),... 43 (100).

4β-Hydroxyclavulara-1(15),17-diene (**2**). $C_{20}H_{32}O$; m.p. 107–110°; [α] +51° (c = 0.554; $CHCl_3$); IR (KBr): ν_{OH} 3360 cm^{-1}, δ = CH_2 885 cm^{-1}; NMR: 0.72 and 0.97 (3H s, $^{16}CH_3$ and $^{20}CH_3$), 1.65 (3H bs, $^{19}CH_3$), 3.28 (1H dd, J = 4.5 and 11, ^{4}CH), 4.70 (4H m, $^{15}CH_2$ and $^{18}CH_2$); MS: 288 (14, M^+), 273 (4, $M^+ - CH_3$), 270 (2, $M^+ - H_2O$), 255 (3, $M^+ - CH_3 - H_2O$), 245 (7, $M^+ - 43$), 232 (4, $M^+ - 56$),... 79 (100).

3α,4β-Dihydroxyclavulara-1(15),17-diene (**3**). $C_{20}H_{32}O_2$; m.p. 78–80°; [α] +51° (c = 0.605; $CHCl_3$); IR (KBr): ν_{OH} 3340 cm^{-1}, δ = CH_2 880 cm^{-1}; NMR: 0.73 and 0.98 (3H s, $^{16}CH_3$ and $^{20}CH_3$), 1.67 (3H bs, $^{19}CH_3$), 2.99 (1H d, J = 9, ^{4}CH), 3.41 (1H m, ^{3}CH), 4.70 (4H m, $^{15}CH_2$ and $^{18}CH_2$); MS: 304 (72, M^+), 289 (20, $M^+ - CH_3$), 286 (53, $M^+ - H_2O$), 271 (22, $M^+ - H_2O - CH_3$), 261 (87, $M^+ - 43$), 248 (43, $M^+ - 56$), 243 (44, $M^+ - 61$),... 81 (100); NMR ^{13}C (in ppm from TMS): 147.7 (s), 147.3 (s), 110.2 (tr), 109.8 (tr), 82.6 (d), 71.6 (d), 55.1 (d), 49.2 (d), 46.9 (d), (44.3, 43.9, 43.9, 43.6, 43.4) two s + three tr, 35.0 (tr), 27.7 (tr), 25.2 (tr), 18.8 (q), 18.8 (q), 10.7 (q).

1α-Hydroxy-4β-acetoxyclavular-17-ene (**4**). 50 mg of **1** treated with 2 ml of pyridine and 1 ml of acetic anhydride yielded 52 mg of **4** which are purified by silica gel column chromatography (eluent: hexane–acetone 9:1). Oil; IR (film): ν_{OH} 3500 cm^{-1}, ν_{CO} 1730 cm^{-1}, δ = CH_2 880 cm^{-1}; NMR: 0.93, 0.99 (3H s, $^{16}CH_3$ and $^{20}CH_3$), 1.15 (3H s, $^{15}CH_3$), 1.67 (3H bs, $^{19}CH_3$), 2.02 (3H s, $OCOCH_3$), 4.47 (1H m, ^{4}CH), 4.67 (2H bs, $^{18}CH_2$); MS: 348 (9, M^+), 333 (3), 330 (5), 305 (7), 292 (9), 288 (14), 273 (11), 270 (12), 266 (6), 255 (8),... 107 (100).

1α-Hydroxy-4-oxoclavular-17-ene (**5**). 40 mg of **1** dissolved in 5 ml of acetone are treated with 5 drops of Jones reagent[11] at room temp. After 5 min the excess of reagent is destroyed by adding 2 ml of MeOH. After usual work up and purification by silica gel column chromatography, 25 mg of **5** are isolated (eluent: hexane–acetone 9:1). Oil; IR (KBr): ν_{OH} 3480 cm^{-1}, $\nu_{C=O}$ 1695 cm^{-1}, δ = CH_2 887 cm^{-1}; NMR: 0.92 (3H s, $^{20}CH_3$), 1.13 (3H s, $^{16}CH_3$), 1.34 (3H s, $^{15}CH_3$), 1.66 (3H bs, $^{19}CH_3$), 4.68 (2H bs, $^{18}CH_2$); MS: 304 (40, M^+), 289 (20), 286 (15), 271 (9), 261 (23), 244 (20), 203 (38),... 99 (100).

4β-Acetoxyclavulara-1(15),17-diene (**7**). Treatment of 50 mg of **2** with 3 ml of the mixture pyridine/Ac_2O 2:1 at room temp. for 24 hr, yielded 45 mg of **7** which are purified by silica gel chromatography (eluent: hexane–acetone 98:2). Oil; [α] +71° (c = 0.28, $CHCl_3$); IR (film): $\nu_{C=O}$ 1740 cm^{-1}, δ = CH_2 885 cm^{-1}; NMR: 0.83 and 0.97 (3H s, $^{16}CH_3$ and $^{20}CH_3$), 1.67 (3H bs, $^{19}CH_3$), 2.03 (3H s, $OCOCH_3$), 4.70 (5H m, $^{4}CH + {}^{15}CH_2 + {}^{19}CH_2$); MS: 330 (22, M^+), 315 (3), 287 (8), 270 (57), 255 (38), 227 (33), 201 (30),... 159 (100).

Conversion of **4** *into* **7**. 50 mg of **4** dissolved in 2 ml of anhyd. pyridine are treated with 0.1 ml of $POCl_3$ and stirred for 8 hr at room temp. After usual work up and purification by silica gel column chromatography (eluent: hexane–acetone 98:2) 41 mg of **7**, identical in all respects ([α], tlc, GC, IR, NMR, MS) with the sample obtained from the acetylation of **2**, are isolated.

4-Oxoclavulara-1(15),17-diene (**8**). Using the procedure described for the oxidation of **1** into **5** by Jones reagent 50 mg of **2** yielded, after purification by silica gel column chromatography (eluent: hexane–acetone 9:1), 42 mg of **8**. Oil; IR (film): $\nu_{C=O}$ 1710 cm^{-1}, δ = CH_2 890 cm^{-1}; UV: end absorption; NMR: 0.95 and 1.03 (3H s, $^{15}CH_3$ and $^{20}CH_3$), 1.67 (3H bs, $^{19}CH_3$), 2.53 (4H s, $^{2}CH_2$ and $^{3}CH_2$), 4.70 (2H bs, $^{18}CH_2$), 4.90 and 5.03 (1H bs, $^{15}CH_2$); MS: 286 (76, M^+), 271 (33), 243 (48),... 93 (100). The intensity of the broad singlet of about 4H present in the NMR spectrum of **8** at 2.53 ppm and attributed to the superposition of the signals of $^{2}CH_2$ and $^{3}CH_2$, is highly reduced in the spectrum of 3,3-^{2}H-4-oxoclavulara-1(15),17-diene (M^+ 288) obtained by

refluxing **8** (40 mg) during 24 hr in 4 ml of D_2O-dioxane 1:1 in presence of 200 mg of K_2CO_3.

3α,4β-Diacetoxyclavulara-1(15),17-diene (**9**). Treatment of 100 mg of **3** with 5 ml of the mixture pyridine/Ac_2O 2:1 at room temp. for 24 hr yielded, after purification by silica gel column chromatography (eluent: hexane–acetone 95:5), 96 mg of **9**. Oil; IR (film): $\nu_{C=O}$ 1750 cm^{-1}; $\delta = CH_2$ 890 cm^{-1}; UV: end absorption; NMR: 0.88 and 0.98 (3H s, $^{16}CH_3$ and $^{20}CH_3$), 1.67 (3H bs, $^{19}CH_3$), 2.00 and 2.05 (3H s, 2×$OCOCH_3$), 4.8 (6H m, $^3CH + ^4CH + ^{15}CH_2 + ^{18}CH_2$); MS: 388 (2, M^+), 373 (2), 354 (1), 345 (3), 328 (35), 315 (4), 286 (28), 268 (88), 253 (38), 243 (13), 225 (25),... 84 (100).

Cleavage of **3** *by sodium periodate.* To 50 mg of **3** in suspension in 30 ml of the mixture MeOH/H_2O 9:1 is added 70 mg of $NaIO_4$ dissolved in 20 ml of the same solvent. The mixture is stirred at room temp. during 40 min. After usual work up, 53 mg of an unstable compound (**10**), homogeneous by tlc, is obtained. On attempted purification of **10** by silica gel column chromatography (eluent: hexane–acetone 8:2) 21 mg of the rearranged product **11** is isolated. IR (KBr): ν_{OH} 3540 cm^{-1}, $\nu_{C=O}$ 1660 cm^{-1}, $\delta = CH_2$ 890 cm^{-1}; UV (CH_3OH): λ_{max} 257.5 (11.600); NMR: 0.93 (6H s, $^{16}CH_3$ and $^{20}CH_3$), 1.67 (3H bs, $^{19}CH_3$), 2.07 (3H bs, $^{15}CH_3$), 3.68 (1H bs, disappearing on treatment with D_2O), 4.53 (1H m, ^4CH), 4.69 (2H bs, $^{18}CH_2$), 10.02 (1H s, CHO); MS: 302 (68, M^+), 287 (14), 284 (18), 273 (5), 269 (20), 256 (19), 241 (14),... 135 (100).

Monoacetate of **11**; oil; IR ($CHCl_3$): $\nu_{C=O}$ 1730 and 1670 cm^{-1}, $\delta = CH_2$ 890 cm^{-1} NMR: 0.93 (6H s, $^{16}CH_3$ and $^{20}CH_3$), 1.65 (3H bs, $^{19}CH_3$), 2.07 (3H bs, $^{15}CH_3$), 2.10 (3H s, $OCOCH_3$), 4.67 (2H bs, $^{18}CH_2$), 5.80 (1H m, ^4CH), 9.60 (1H s, CHO); MS: 344 (14, M^+), 302 (35), 301 (56), 287 (16), 284 (39), 273 (5), 269 (21),... 43 (100).

Reduction of **10** *into* **12**. When 45 mg of crude **10** dissolved in 8 ml of MeOH are treated with 150 mg of $NaBH_4$ at room temp. for 30 min., 22 mg of the diol **12** are obtained. IR (KBr): ν_{OH} 3320 cm^{-1}, $\delta = CH_2$ 890 cm^{-1}; NMR: 0.97 (6H s, $^{16}CH_3$ and $^{20}CH_3$), 1.68 (3H bs, $^{19}CH_3$), AB system centered at 3.30 ppm (δ_A = 3.41, δ_B = 3.19, J_{AB} = 11 Hz), 3.77 (2H t, J = 7, $^3CH_2$), 4.70 and 4.93 (bs, 2H each, $^{18}CH_2$ and $^{15}CH_2$); MS: 306 (1, M^+), 288 (2), 270 (17), 255 (10), 227 (7),... 93 (100).

Catalytic hydrogenation of **3**. Catalytic hydrogenation (EtOH/PtO_2) of 49 mg of **3**, at room temp and 760 mm/Hg during 45 min, yielded after extraction and purification by silica gel column chromatography (eluent: hexane–acetone 8:2) 42 mg of **13**. IR (KBr): ν_{OH} 3310 cm^{-1}; NMR (pyridine): 0.82 (6H tr, J = 7, $^{18}CH_3$ and $^{19}CH_3$), 0.93 and 1.20 (s, 3H each, $^{16}CH_3$ and $^{20}CH_3$), 1.01 (3H d, J = 7, $^{15}CH_3$), 3.22 (1H d, J = 9, ^4CH), 4.06 (1H m, ^3CH); MS: 308 (3, M^+), 290 (11), 275 (3), 272 (4), 259 (3), 247 (3), 234 (15), 233 (13), 205 (5),... 84 (100).

Cleavage of **13** *by* $NaIO_4$. Treatment of **13** with $NaIO_4$ using the procedure described above, yielded compound **14**. Oil; IR (film): $\nu_{C=O}$ 1730 cm^{-1} ν_{CHO} 2720 cm^{-1}; NMR: 0.82 (6H tr, J = 7, $^{18}CH_3$ and $^{19}CH_3$), 0.92 and 1.12 (s, 3H each, $^{16}CH_3$ and $^{20}CH_3$), 0.97 (3H d, J = 8, $^{15}CH_3$), 9.1 (1H s, ^4CH), 9.4 (1H m, ^3CH); MS: 306 (1, M^+), 291 (1), 288 (1), 277 (5), 259 (10), 245 (2), 234 (4),... 84 (100).

4β-Hydroxy-3α-tosyloxyclavulara-1(15),17-diene (**15**). To 247 mg of **3** in 10 ml of anhyd. pyridine was added at 0° a solution of 3.2 g of *p*-toluenesulfonylchloride in 5 ml of anhyd pyridine, and the mixture was stirred at room temp. during 20 hr. After usual work-up, 345 mg of crude mixture were obtained which were purified by silica gel column chromatography (eluent: $CHCl_3$–C_6H_6 7:3) to yield 230 mg of oily monotosylate **15**. IR (film): ν_{OH} 3580 cm^{-1}, $\nu_{C=C}$ 1645 and 1600 cm^{-1}, ν_{SO_2} 1360 and 1180 cm^{-1}; NMR: 0.75 and 0.95 (s, 3H each, $^{16}CH_3$ and $^{20}CH_3$), 1.65 (3H s, $^{19}CH_3$), 2.45 (3H s, CH_3-Ar), 3.27 (1H dd, J = 9 and 3, collapsing into a doublet, J = 9 Hz on D_2O treatment, ^4CH), 4.40 (1H m, ^3CH), 4.75 (4H m, $^{15}CH_2$ and $^{18}CH_2$), 7.57 (4H m, aromatic CH); MS: 458 (3.5, M^+), 443 (1.5), 415 (2.5), 402 (2), 286 (1),... 81 (100).

LAH reduction of **15** *into* **2** *and* **16**. 80 mg of **15** in 10 ml of anhyd. THF were treated with 150 mg of LAH at reflux during 4 hr. Usual work-up affords 72 mg of a mixture (essentially 2 compounds by tlc) which was separated on a silica gel column (eluent: hexane–AcOEt 8:2) affording 40 mg of **2** (identified by tlc, IR, NMR, MS, glc and [α]) and 15 mg of **16**. IR (KBr): ν_{OH} 3450 cm^{-1}, $\nu_{C=C}$ 1645 cm^{-1}, $\delta = CH_2$ 885 cm^{-1}; NMR: 0.96 and 1.00 ppm (3H each, s, $^{16}CH_3$ and $^{20}CH_3$), 1.63 (3H bs, $^{19}CH_3$), 2.35 (2H m, $^2CH_2$), 4.00 (1H m, ^3CH), 4.77 (4H m, $^{15}CH_2$ and $^{18}CH_2$); MS: 288 (7.5, M^+), 273 (6), 270 (1), 257 (2), 245 (2.5),... 81 (100).

3β,4β-Oxidoclavulara-1(15),17-diene (**17**). 63 mg of **15** were treated during 0.5 hr with 13 ml of a saturated methanolic solution of K_2CO_3. Usual work-up and silica gel column chromatography afford 34 mg of epoxide **17**. IR (film) no ν_{OH}; $\nu_{C=C}$ 1650 cm^{-1}, $\delta_{C=CH_2}$ 890 cm^{-1}; NMR: 0.91 and 0.96 (s, 3H each, $^{16}CH_3$ and $^{20}CH_3$), 1.67 (3H bs, $^{19}CH_3$), 2.7 (3H m, ^4CH and $^2CH_2$), 3.23 (m, ^3CH), 4.7 (3H bs) and 4.91 (1H bs, $^{15}CH_2$ and $^{18}CH_2$); MS: 286 (3.5, M^+), 271 (2), 243 (3.5),... 91 (100).

Mild LAH reduction of **15** *into* **2**, **16** *and* **17**. 70 mg of **15** in 10 ml of anhyd. THF were treated during 12 hr under stirring with 150 mg LAH affording, after usual work-up, a mixture of 4 components. They were identified to **17**, **2**, **16** and **3** by comparison of their Rf in tlc with those of authentic specimens.

LAH reduction of **17** *into* **2** *and* **16**. Epoxide **17** treated with LAH under reflux in anhyd. THF affords the mixture of monoalcohols **2** and **16**, already obtained from **15** by the same reaction (*vide supra*).

Acknowledgements—This work was made possible by the Leopold III Foundation and the Franqui Foundation. We gratefully acknowledge their support. We thank Dr. J. Verseveldt for identification of the animal, Dr. R. Ottinger for valuable discussions and Dr. D. Zimmermann for the ^{13}C NMR spectrum. We are most indebted towards Jean Pierret and Syd Johnson who participated in all phases of the work at Laing Island with their usual competence and enthusiasm.

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