ARCTOLIDE-REVISION OF ITS STEREOSTRUCTURE AND DETERMINATION OF ABSOLUTE CONFIGURATION. DETAILED ^{1}H AND ^{13}C NMR ANALYSIS*

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Dedicated to Prof. G. Snatzke on the occasion of his 60th birthday.

The stereostructure of arctolide — a sesquiterpenic lactone isolated from *Arctotis grandis* — has been revised on the basis of detailed ^{1}H and ^{13}C NMR analysis and detection of intramolecular hydrogen bonding by IR spectroscopy. Its absolute configuration, represented by formula *Ia*, was determined from the CD spectra. The scope and limitation of modern 1D- and 2D-NMR techniques in obtaining parameters utilizable for structure determination of natural compounds have been verified.

In the year 1977 we described the isolation of arctolide[1] (the main sesquiterpenic lactone in the aereal part of species *Arctotis grandis* THUNB., family *Compositae*, tribe *Arctotae*), determined its constitution and suggested its relative configuration, represented by formula *I*. The suggested structure *I* was derived from the ^{1}H NMR spectrum (100 MHz, CW-mode) of arctolide and its trichloroacetylcarbamoyl derivative prepared by in situ acylation (the TAI-method[2,3]). The assignment of relative configuration at the carbon atoms C-1, C-5, C-7 and C-8 was based on the following observations (for a detailed discussion see ref.[1]). *trans*-Annelation the of the γ-lactone ring was shown by the high values of $^{3}J(7, 8)$ ($\approx$ 10 Hz) and of the allylic coupling constants $^{4}J(7, 13)$ (3·5 Hz) and $^{4}J(7, 13')$ (3·0 Hz), in accord with the lactone rule[4,5]. The *cis*-fusion of the five-membered and the seven-membered homocycles was indicated by the large paramagnetic TAI-acylation shift of the H-1 proton

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* Part CCXCVII in the series On Terpenes; Part CCXCVI: Collect. Czech. Chem. Commun. *52*, 1786 (1987).
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(1·16 ppm), obviously due to synperiplanar or synclinal arrangement of H-1 and the hydroxyl on C-5. The *cis*-annelation was also supported by the TAI-acylation--induced conformational changes, manifested by TAI-acylation shifts of the H-8, H-9 and H-9′ proton signals $(-0·10, -0·16$ and $0·16$ ppm, respectively). The *cis*--relation of the hydroxyl and the H-7 hydrogen was derived from the coupling constants $^3J(6, 7)$ and $^3J(6′, 7)$ (1 and 10·5 Hz, respectively) and from the marked TAI-acylation shift of the H-6 proton (0·66 ppm). Substantially less convincing arguments were available for configurational assignment of the oxygen functionalities on C-3 and C-10. It has been stressed that the suggested structure *I* represents only one of the several stereostructural possibilities.

Recently, we isolated sufficient amounts of arctolide and several other minor structurally related sesquiterpenic lactones from *Arctotis grandis* THUNB.[6,7]. This, together with the contemporary experimental possibilities of NMR spectroscopy, prompted us to tackle the still incompletely solved structural problem. Our present communication concerns the detailed structural analysis of arctolide, using modern NMR methods and IR and CD spectra of suitable derivatives.

I

I a, R = H
I b, R = CONHCOCCl₃
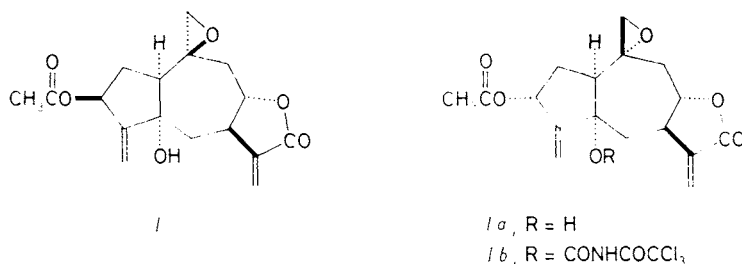
RESULTS AND DISCUSSION

As seen from molecular models, the presence of *cis*-fused five- and seven-membered homocyclic rings in the molecule of arctolide makes the system somewhat flexible, with several possible conformations. The determination of the preferred conformation of arctolide, necessary for the relative configurational assignment, is further complicated by the presence of quaternary carbon atoms in positions 4, 5 and 10. Our recent ^{1}H NMR measurements of arctolide and its TAC-derivative at 200 MHz (Fig. 1) afforded more accurate values of chemical shifts and coupling constants of all the protons (Table I).

The partial overlap of H-3, H-13′ (δ 5·60) and H-1, H-6′ and H-9′ (δ 1·9) proton signals was eliminated in the proton J-resolved 2D-NMR spectrum (Fig. 2), which allowed an unequivocal identification of the multiplets of all protons and confirmed the $J(H, H)$ values.

The spin–spin interactions were assigned on the basis of ^{1}H–^{1}H homocorrelated 2D-NMR spectrum (Fig. 3) and the obtained structural assignment does not differ from that published previously[1]. From the standpoint of conformation analysis, the most important new contribution is the observed four-bond long-range coupling between the H-1 and H-6 protons (1·1 Hz). This indicates an approximately planar zig-zag arrangement of bonds in the segment (H-1)—(C-1)—(C-5)—(C-6)—(H-6α), limiting thus substantially the number of possible conformations. With the sufficiently proven *cis*-annelation of the five- and seven-membered rings and *trans*-annelation of the γ-lactone ring, the only possible pseudorotation is that about the (C-9)—(C-10) bond between the two limit conformations A and B of the seven-membered ring. Of them, only the form A is compatible with the observed values of $J(8, 9)$ and $J(8, 9')$ (7·4 and 9·4 Hz, respectively). These values, together with those of $J(6, 7)$ and $J(6, 7')$ (1·5 and 10·9 Hz), lead to configurational assignment of methylene proton signals in positions 6 and 9 (Table I). The five-membered ring
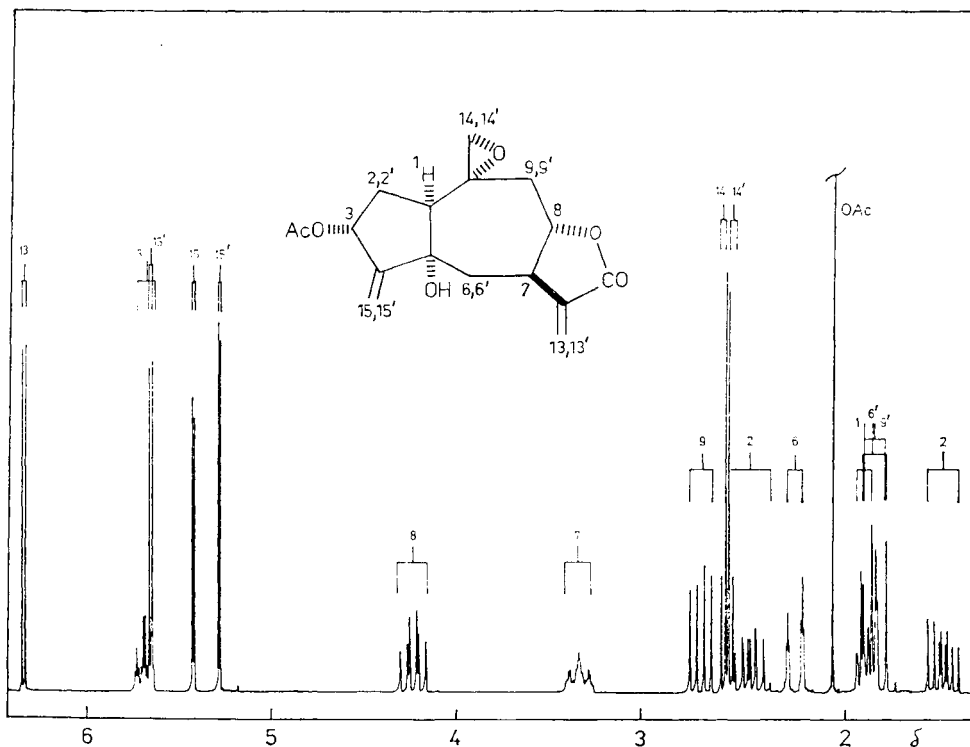

FIG. 1
Proton NMR spectrum of arctolide

can theoretically assume conformations of the type ^2E, E_1, E^5 and E_4 or the inter-mediate twist forms. The coupling constants $J(1, 2)$, $J(1, 2')$, $J(2, 3)$ and $J(2', 3)$ (7·7, 11·8, 8·6 and 6·5 Hz, respectively) do not allow a dependable simultaneous determination of the ring conformation and configuration at the C-3 carbon atom. We tried therefore to obtain further stereochemical information from measurements of nuclear Overhauser effect (NOE).
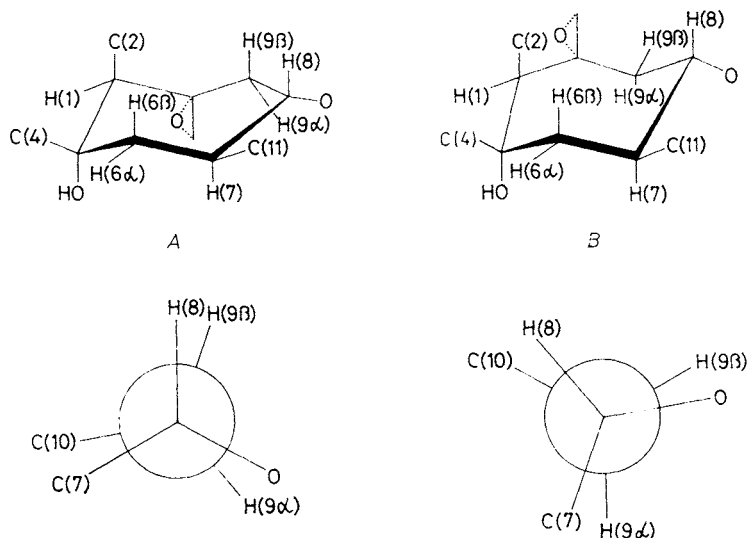
The proton 2D-NOE spectrum of arctolide is shown in Fig. 4. Comparison with its ^{1}H–^{1}H homocorrelated 2D-NMR spectrum (Fig. 3) identifies the so-called "pure NOE peaks", indicating protons without mutual spin–spin coupling but with a significant contribution to the dipolar relaxation, and thus spatially close. As expected, strong NOE peaks occur between the exomethylene protons H-13 and H-13', and H-15 and H-15'. Both the H-13 and H-13' protons exhibit a weaker NOE with the H-6α proton. More surprising is the observed NOE peak of the exomethylene protons H-15 and H-15' with the H-2' proton, the H-15' atom having moreover a weaker NOE peak with the H-2 proton. However, as concerns the configuration at C-3, the most important NOE peak is that between the hydrogen atoms of the acetoxy group and one of the oxirane hydrogens on C-14. These atoms can be sufficiently close only when the acetoxy group has the 3α-configuration. Moreover, only the 3α--acetoxy group can form an intramolecular hydrogen bond with the 5α-hydroxyl bringing thus the acetoxy protons close to H-14. Surprisingly, no NOE peak was observed between H-8 and H-2 protons and the overlap of the H-1, H-6' and H-9' signals did not allow any unequivocal proof of the expected NOE peak between the H-8 and H-6β protons.

In our further investigation we intended to use arctolide for checking the hitherto little studied potentialities of ^{13}C NMR data, particularly the coupling constants $J(C, H)$, for solving stereochemical problems in sesquiterpenic lactones. Naturally, an unequivocal assignment of all carbon atoms in the ^{13}C NMR spectrum was the necessary condition. The most readily accessible data were obtained from the proton--decoupled spectrum of arctolide (Fig. 5) which exhibited all the 17 expected signals. Only the methyl carbon atom in the acetoxy group on C-17 (δ 21·08) can be unequivocally assigned on the basis of chemical shift, whereas for the carbonyl, exomethylene and other carbon atoms several alternative assignments existed. Further usual step in the assignment of carbon atoms is an experiment distinguishing carbon atoms of the CH_3, CH_2, CH and C types. We have compared the following accessible methods: *a*) observation of residual $^1J(C, H)$ constants in the off-resonance proton--decoupled ^{13}C NMR spectrum; *b*) distinction between carbon atoms with even and odd number of directly attached protons according to the positive and negative amplitude of the signal in the so-called APT spectrum; *c*) edited spectra of the

TABLE I

Proton NMR parameters of arctolide (*Ia*) in deuterochloroform

Proton (config.)	$\delta(H)$	$\Delta\delta(H)^a$	$J(H, H)^b$					
H-1 (α)	1·91 bdd	1·16	$J(1, 2)$	7·7;	$J(1, 2')$	11·8;	$J(1, 6)$	1·2
H-2 (α)	2·52 ddd	0·01	$J(2, 2')$	13·7;	$J(2, 1)$	7·7;	$J(2, 3)$	8·6
H-2' (β)	1·51 ddd	0·05	$J(2', 2)$	13·7;	$J(2', 1)$	11·8;	$J(2', 3)$	6·5
H-3 (β)	5·65 ddt	0·04	$J(3, 2)$	8·6;	$J(3, 2')$	6·5;	$J(3, 15)$	2·1
			$J(3, 15')$	1·8				
H-6 (α)	2·28 dt	0·60	$J(6, 6')$	15·0;	$J(6, 7)$	1·5;	$J(6, 1)$	1·2
H-6' (β)	1·88 dd	0·03	$J(6', 6)$	15·0;	$J(6', 7)$	10·9		
H-7 (α)	3·40 dtt	−0·05	$J(7, 6)$	1·5;	$J(7, 6')$	10·9;	$J(7, 8)$	9·8
			$J(7, 13)$	3·5;	$J(7, 13')$	3·1		
H-8 (β)	4·27 ddd	−0·12	$J(8, 7)$	9·8;	$J(8, 9)$	7·4;	$J(8, 9')$	9·4
H-9 (α)	2·77 dd	−0·20	$J(9, 9')$	14·6;	$J(9, 8)$	7·4		
H-9' (β)	1·87 dd	0·17	$J(9', 9)$	14·6;	$J(9', 8)$	9·4		
H-13	6·28 d	0·00	$J(13, 7)$	3·5				
H-13'	5·62 d	0·03	$J(13', 7)$	3·1				
H-14	2·65 d	0·03	$J(14, 14')$	4·3				
H-14'	2·62 d	−0·02	$J(14', 14)$	4·3				
H-15	5·40 d	0·06	$J(15, 3)$	2·1				
H-15'	5·27 d	0·18	$J(15', 3)$	1·8				
OAc	2·09 s	0·01	—					

^{a} TAI-acylation induced chemical shifts; ^{b} absolute values of coupling constants are given.

individual types of carbon atoms, obtained by the so-called DEPT experiment, and
d) measurement of relaxation times T_1.

The drawback of the off-resonance decoupling method (Fig. 6) is a lower sensitivity
(the signal intensity is distributed into the broadened lines of the multiplet) and
sometimes it is also difficult to determine multiplicities of signals with similar
chemical shift (in our case three carbon atoms at about δ 40·0).

The APT experiment (Fig. 7) is much more sensitive and removes complications
with the partial overlap of signals, but does not discriminate between the negative
(CH_3 and CH) and positive (CH_2 and C) signals which have to be distinguished by
other arguments (chemical shift or signal intensity).

All the mentioned shortcomings are overcome by the edited spectra from DEPT
experiment (Fig. 8), at the cost of about four times longer measurement time as
compared with that of the APT experiment. A possible complication of the DEPT
spectra is shown in Fig. 8: the spectrum of CH-carbons exhibits a weak signal at
δ 50·62 which appears again, with higher intensity, in the spectrum of CH_2-carbons
where it actually belongs. The reason is its high value of $^1J(C, H)$, 174·8 Hz (Table II),
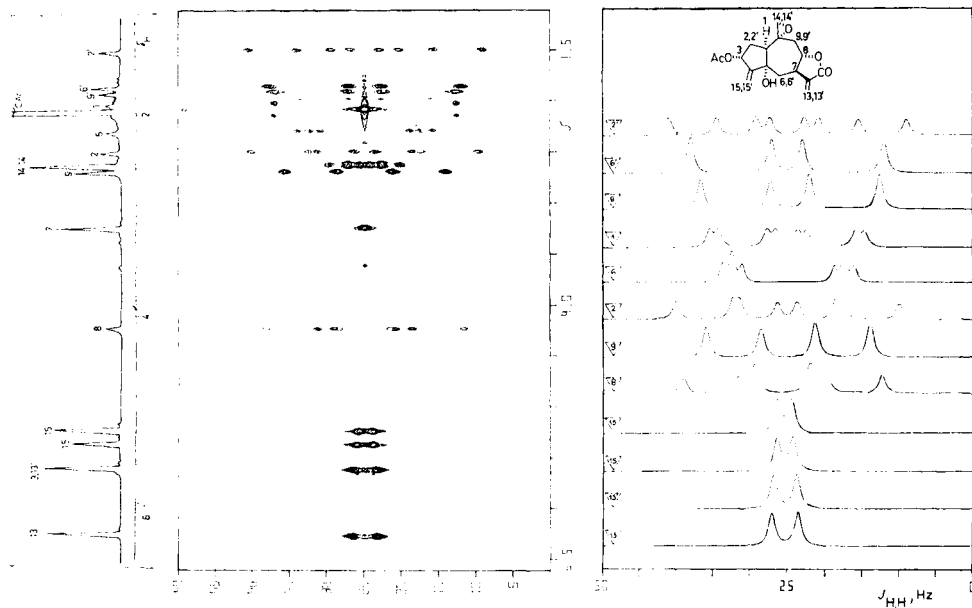which is substantially higher than the mean value used as parameter for the given
experiment.

FIG. 2

Homonuclear proton-*J*-resolved 2D-NMR spectrum of arctolide

The classification of carbon signals according to the number of directly attached protons is also possible using the measurement of relaxation times T_1 (Fig. 9). Although the range of the measured values is very broad $(0.39 - 21.07$ s; see Table II) they can be relatively easily divided into four groups. In accord with the theory, the shortest times T_1 (about 0.5 s) belong to the six CH_2 carbon atoms and the approximately twice longer times T_1 $(\approx 1.0$ s) to the four CH carbon atoms; this indicates an isotropic reorientation of the molecule. It is known that the internal motion of the methyl group increases the T_1 value. In our case, the found value
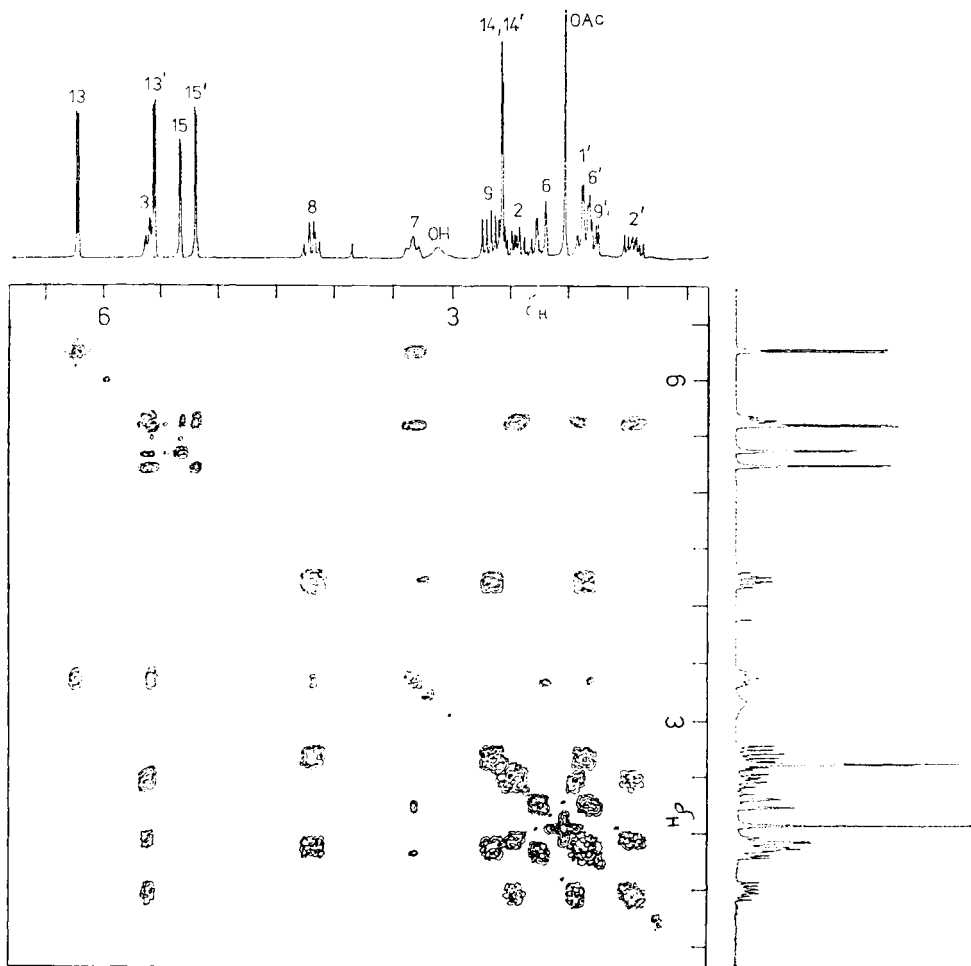

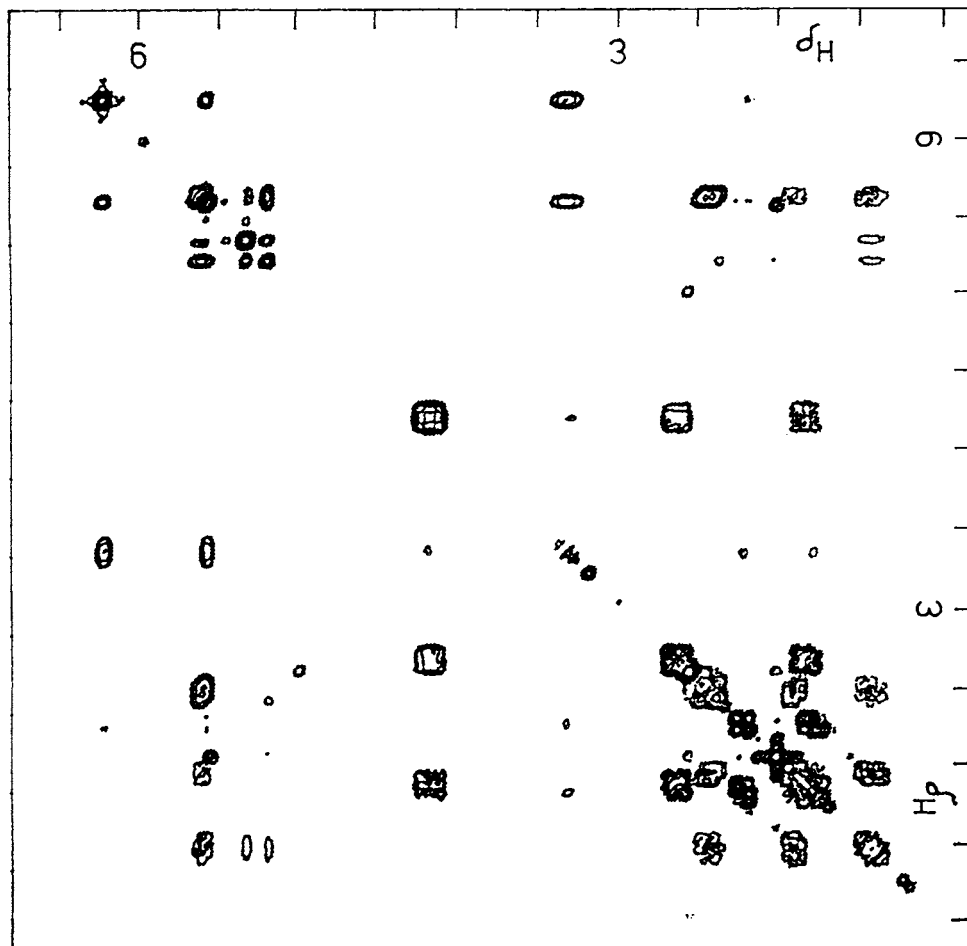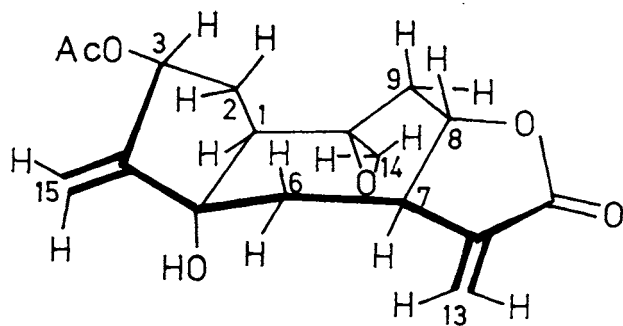FIG. 3

Homocorrelated $^1H-^1H$ 2D-NMR spectrum of arctolide

Fig. 4

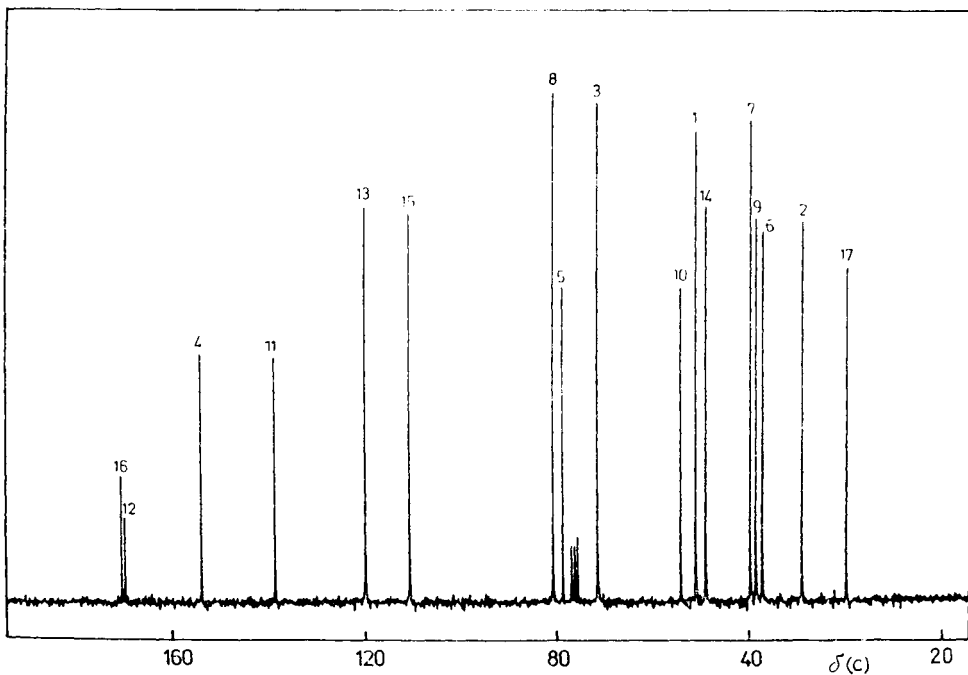Homonuclear ^{1}H–^{1}H NOE 2D-NMR spectrum of arctolide

FIG. 5

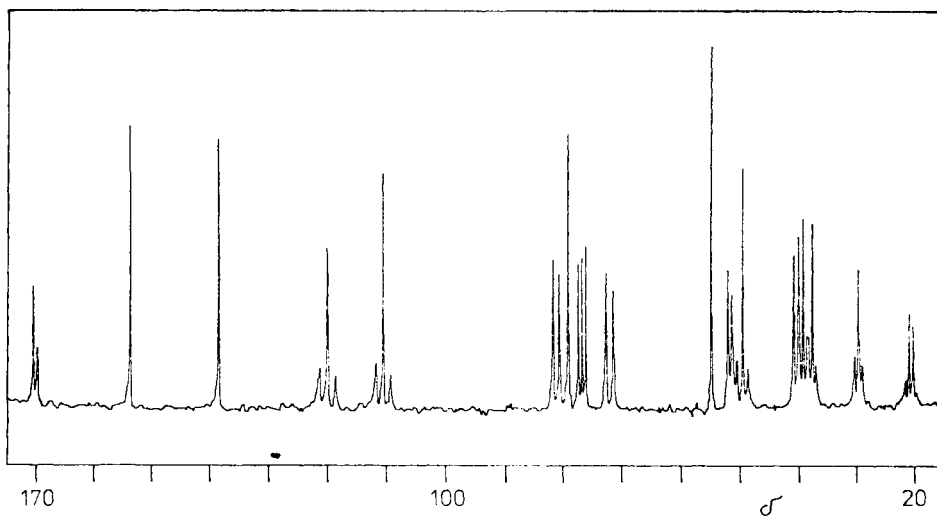Carbon-13 NMR spectrum (broadband proton-decoupling) of arctolide

FIG. 6

Carbon-13 NMR spectrum (off-resonance proton-decoupling) of arctolide

of 3·7 s for the CH_3 carbon atom in the acetoxy group can also be influenced by a higher local mobility of this group. Because of the dominant ^1H–^{13}C dipole–dipole relaxation mechanism, the highest T_1 values are observed, as expected, for the six quaternary carbon atoms $(8-21 s)$ with markedly longer T_1 for both the carbonyl carbon atoms.

In this context, it is worth notice that the TAC-derivative of arctolide has much smaller values of T_1 (three times in average). This is probably due to introduction of the very bulky —$CONHCOCCl_3$ group which results in a slower reorientation of the molecule, longer correlation time τ_c and thus shorter T_1's. This decrease in the T_1 values has one positive consequence of general utility: a shortening of the pulse repetition time in the ^{13}C NMR spectral measurements and a better S/N ratio obtained in the same time interval.

The ^{13}C NMR acylation shifts for the TAC-derivative of arctolide can be utilized for the assignment of carbon atoms in the neighbourhood of the acylated hydroxyl.
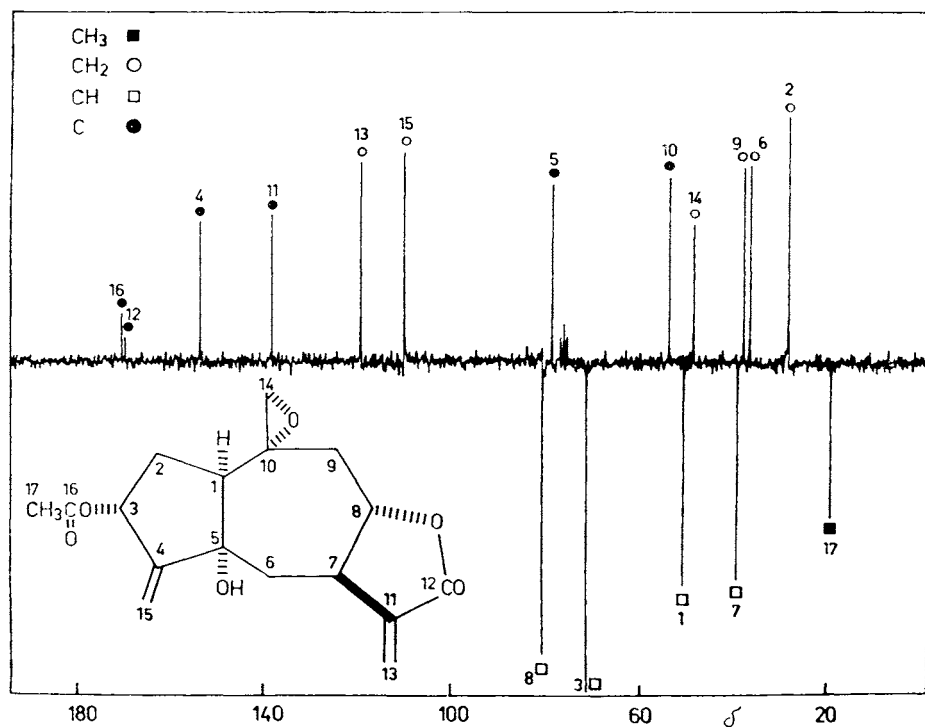

FIG. 7

"Attached proton test" carbon-13 NMR spectrum of arctolide

In accord with the empirical rules[8,9], the downfield shift of 10·60 ppm indicates an α-carbon atom bearing an acylated hydroxyl, i.e. C-5. Upfield shifts of −4·21, −5·15 and −2·52 ppm are observed for the β-carbon atoms, i.e. C-1, C-4 and C-6.

An unequivocal assignment of all the eleven hydrogen-bearing carbon atoms has been achieved using the ^{13}C–^{1}H heterocorrelated 2D-NMR spectrum (Fig. 10). The remaining six quaternary carbon atoms are two carbonyl carbon atoms C-12 and C-16, olefinic carbon atoms C-4 and C-11 and finally the carbon atoms C-5 and C-10, bonded to oxygen atoms. The last-mentioned atoms of the C—O type can be assigned on the basis of chemical shifts: the upfield signal at δ 55·60 should be due to the oxirane carbon atom C-10 whereas the downfield signal at δ 79·61 to the C—OH carbon atom in position 5. Concerning the two olefinic carbon atoms, comparison with ^{13}C NMR data of a series of sesquiterpenic α-exomethylene-γ-lactones shows that the signal at δ 138·75 is due to C-11 whereas the signal at δ 153·88 belongs to C-4. The carbonyl carbon atoms with very similar values δ 169·77 and 170·55 cannot be distinguished by the discussed approach.

To assign these carbon atoms, but first of all to obtain the coupling constants $J(C, H)$, we measured the heteronuclear $J(C, H)$-resolved 2D-NMR spectrum
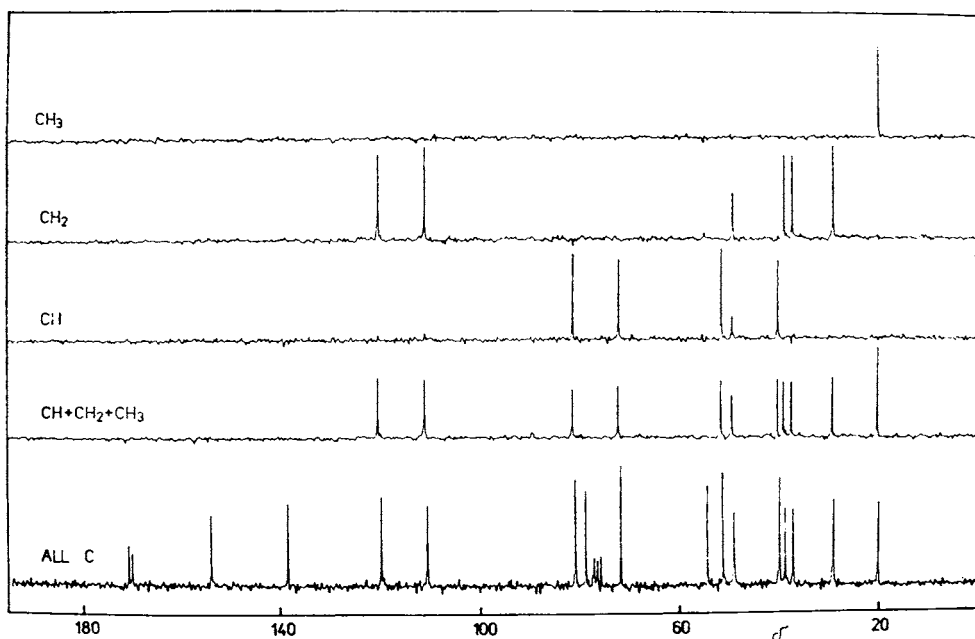

FIG. 8

Edited carbon-13 NMR spectra of CH_3, CH_2, CH and all carbon atoms of arctolide

TABLE II

Carbon-13 NMR parameters of arctolide (*Ia*) in deuterochloroform

Carbon	δ(C)	Type	$T_1{}^a$	$\Delta\delta$(C)^b	^1J(C, H)^c		N^d	^2J(C, H); ^3J(C, H)^c
C-1	52·22	>CH—	0·90 (0·29)	−4·21	J(C-1, H-1)	129·9	(10)	—^{e}
C-2	30·36	—CH$_2$—	0·48 (0·44)	−0·22	J(C-2, H-2)	136·6^f	(2)	J(C-2, H-1) 4·2
					J(C-2, H-2′)	127·8^f		J(C-2, H-3) 2·1
C-3	72·33	>CH—O	0·97 (0·33)	−0·01	J(C-3, H-3)	153·4	(5)	—^{e}
C-4	153·88	>C═	8·00 (2·83)	−5·15	—		(7)	—^{e}
C-5	79·61	>C—O	10·07 (4·17)	10·60	—		(10)	—^{e}
C-6	38·81	—CH$_2$—	0·49 (0·08)	−2·52	J(C-6, H-6)	130·1^f	(3)	J(C-6, H-7) ≈ 3·1; J(C-6, H-1) ≈ 3·1
					J(C-6, H-6′)	126·9^f		J(C-6, H-8) ≈ 3·1
C-7	40·63	>CH—	0·91 (0·25)	0·45	J(C-7, H-7)	130·8	(7)	—^{e}
C-8	81·29	>CH—O	0·97 (0·25)	0·18	J(C-8, H-8)	149·3	(5)	J(C-8, H-7) ≈ J(C-8, H-9) ≈ J(C-8, H-9′) ≈ 5·9
								J(C-8, H-6) 11·6; J(C-8, H-6′) ≈ 5·9
C-9	39·30	—CH$_2$—	0·56 (0·12)	0·46	J(C-9, H-9)	129·8	(5)	—^{e}
					J(C-9, H-9′)	129·8		
C-10	55·60	>C—O	8·09 (2·92)	−1·71	—		(8)	—^{e}
C-11	138·75	>C═	10·01 (3·58)	−0·62	—		(6)	J(C-11, H-7) = J(C-11, H-13) 2·9
								J(C-11, H-13′) 7·8; J(C-11, H-6) ≈ 1·0
								J(C-11, H-6′) ≈ J(C-11, H-8) ≈ 0
C-12	169·77	>C═O	21·07 (18·84)	−0·24	—		(4)	J(C-12, H-13) 6·7; J(C-12, H-13′) 13·8
								J(C-12, H-7) ≈ J(C-12, H-8) ≈ 0
C-13	120·39	═CH$_2$	0·39 (0·14)	0·01	J(C-13, H-13)	163·6^f	(1)	J(C-13, H-7) 4·5
					J(C-13, H-13′)	160·9^f		
C-14	50·62	—CH$_2$—O	0·51 (0·08)	−1·01	J(C-14, H-14)	174·8	(3)	J(C-14, H-1) 4·5; J(C-14, H-9) 3·5
					J(C-14, H-14′)	174·8		J(C-14, H-9′) 1·0
C-15	111·41	═CH$_2$	0·41 (0·11)	2·43	J(C-15, H-15)	158·9	(1)	J(C-15, H-3) 3·4
					J(C-15, H-15′)	158·9		
C-16	170·55	>C═O	17·80 (4·33)	−0·42	—		(4)	J(C-16, H-17) 6·8 (3×); J(C-16, H-3) 3·1
C-17	21·08	—CH$_3$	3·74 (0·98)	−0·14	J(C-17, H-17)	129·6 (3×)	(0)	—

^{a} The T_1 values of TAC-derivative *Ib* are given in parentheses; ^{b} TAI-induced acylation shifts (in ppm); ^{c} absolute values of J(C, H) are given; ^{d} the numbers of expected geminal and vicinal J(C, H) couplings are given in parentheses; ^{e} values of ^nJ(C, H) could not be determined; ^{f} values of J(C, H) can be mutually interchanged.
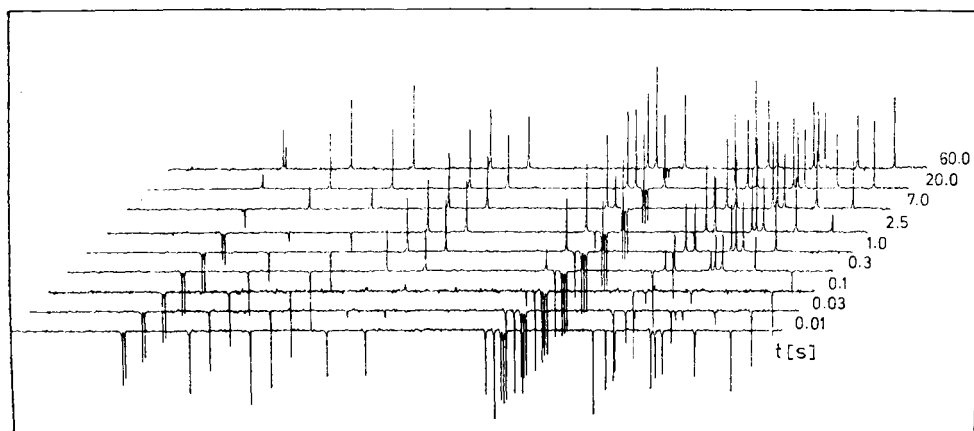
FIG. 9

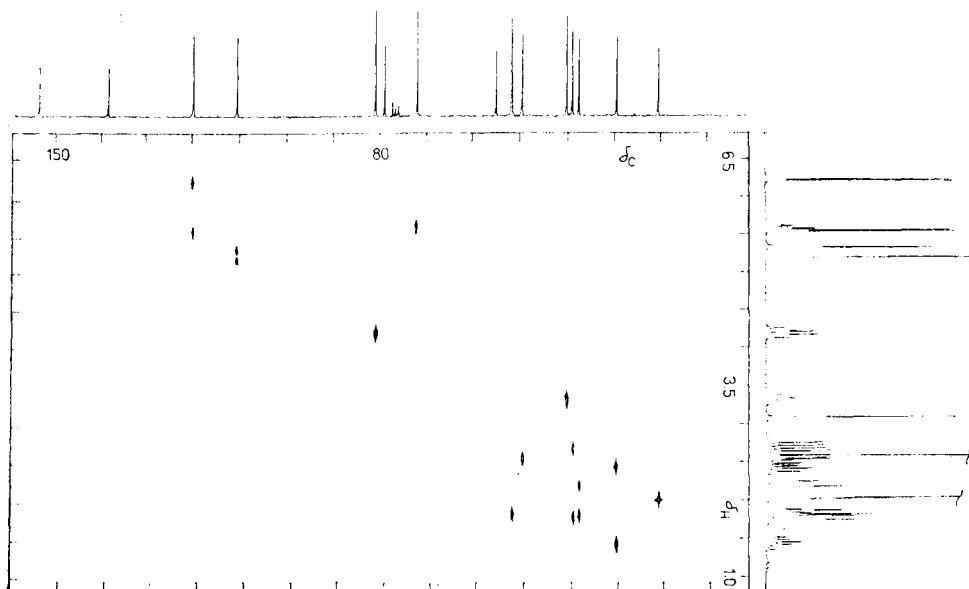Measurement of carbon-13 relaxation times $T1$ (inversion-recovery technique)

FIG. 10

Heteronuclear ^{13}C-^{1}H Correlated 2D-NMR spectrum of arctolide

(Fig. 11) and the proton-coupled ^{13}C NMR spectrum of arctolide (Fig. 12). Both the spectra clearly show the potentialities, as well as limitations, of this approach. The direct coupling constants $^1J(C, H)$ can be obtained from both spectra relatively easily. The found values (Table II) for sp^3 carbon types CH, CH$_2$ and CH$_3$ are about 130 Hz, for CH—O carbon atoms about 150 Hz (except for the oxirane carbon C-14 with an extremely high value of 175 Hz) and for exomethylene sp^2 carbon atoms about 160 Hz; the values are thus in accord with the known effects of hybridization and substitution[10]. A substantially more complex problem is the extraction of the so-called long-range couplings $J(C, H)$ across two or three bonds. The observed fine structure of signals in proton-coupled ^{13}C-NMR spectra depends on the number and magnitude of these couplings. As follows from the structure of arctolide, the number of couplings $(^2J(C, H) + {}^3J(C, H))$ for the individual carbon atoms ranges from zero to ten (Table II). It is therefore not very surprising that
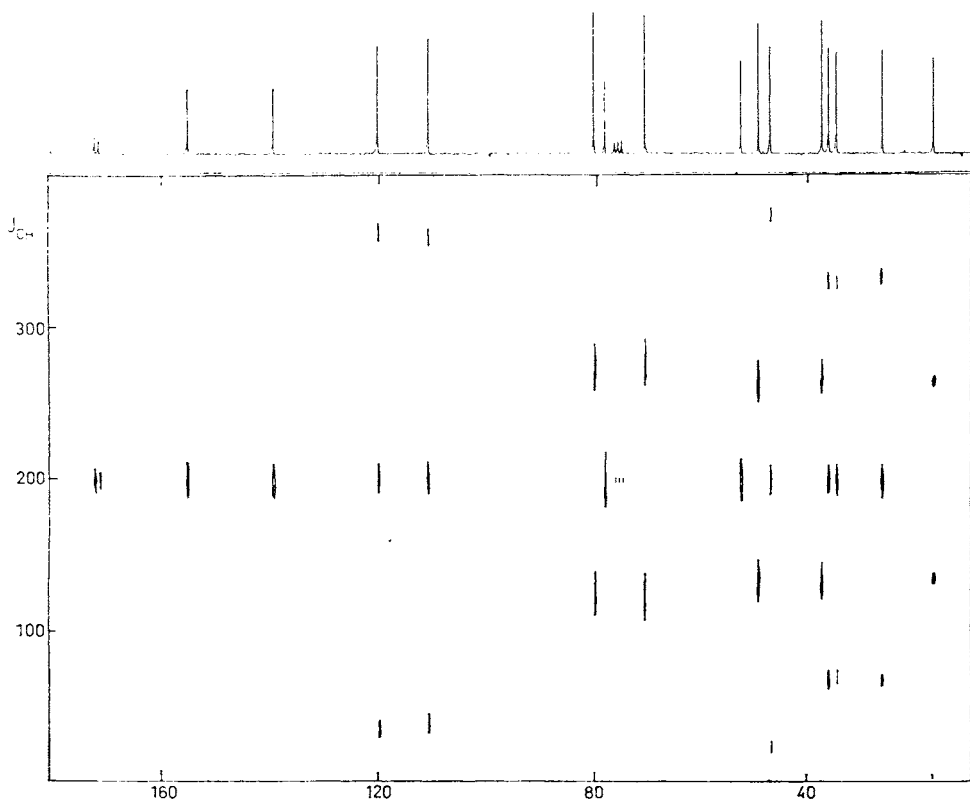

FIG. 11

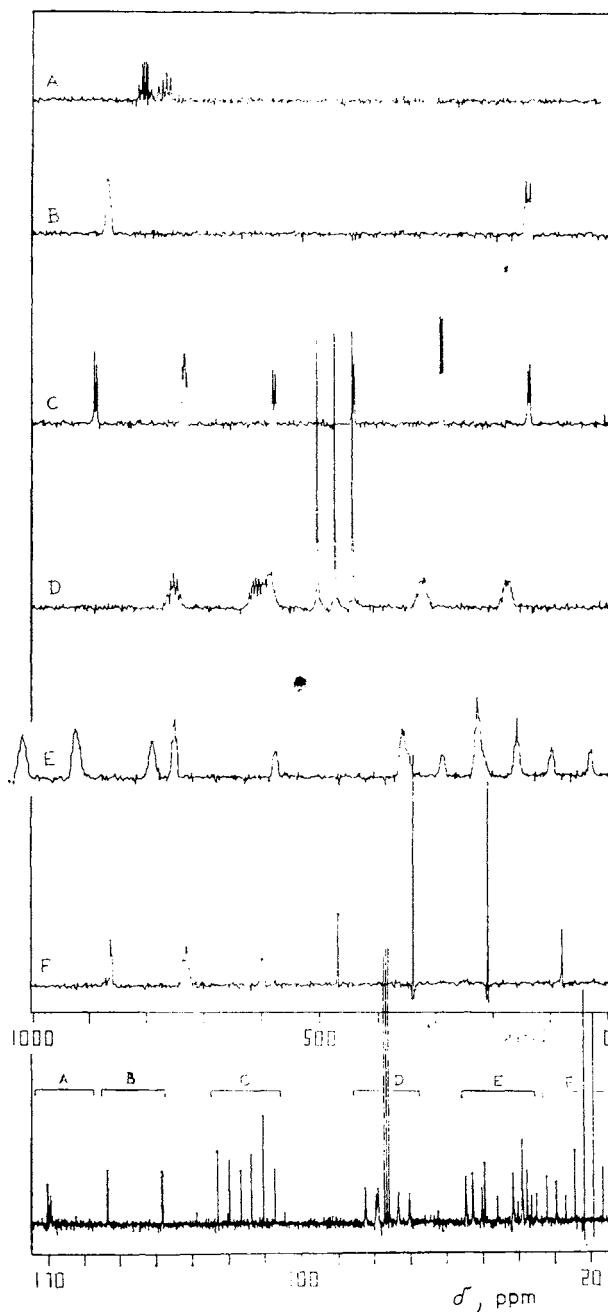Heteronuclear $J(^{13}C, {}^1H)$-resolved 2D-NMR spectrum of arctolide

FIG. 12

Proton-coupled carbon-13 NMR spectrum of arctolide: full spectrum (below) and expansions of parts A—F (above)

multiplets of only some carbon atoms are analyzable. Although the 2D-J(C, H)--resolved spectrum (Fig. 11) eliminates the overlap of some multiplets occuring in the 1D-spectrum (Fig. 12), problems with the fine structure resolution remain. Complete analyses were achieved only for multiplets of C-2, C-6, C-8, C-11, C-13, C-14, C-15, C-16 and C-17, with five or less long-range couplings (their values are given in Table II) whereas the remaining carbon atoms afforded insufficiently resolved multi-plets even in selective proton-decoupling experiments. Using the J(C, H) values, it was at last possible to assign the carbonyl carbon atoms. The doublet of quartets at δ 170·55 must belong to C-16 of the acetoxy group with ^2J(C-16, H-17) = 6·8 Hz (3 ×) and ^3J(C-16, H-3) = 3·1 Hz whereas the doublet of doublets at δ 169·77 is ascribed to C-12, with ^3J(C-12, H-13) = 6·7 Hz and ^3J(C-12, H-13') = 13·8 Hz, its two further ^3J(C, H) with H-7 and H-8 being apparently close to zero (in accord with the expected dihedral angles of about 90°). Also other available values of ^3J(C, H) agree qualitatively with the suggested conformation A of the seven-mem-bered homocycle and with the *trans*-annelated γ-lactone ring. Important for deter-mination of the preferred conformation of the five-membered homocycle is the found value of ^3J(C-15, H-3), amounting to 3·4 Hz. Its magnitude indicates a ^2E conforma-tion with dihedral angle Φ (C-15, H-2) of about 30° (for ^3J(C, H) $= f(\Phi)$ see ref.[11]) because for the other theoretically possible types $- E_1$, E^5 and E_4 $-$ this angle is 60 $-$ 100°. Also the values of ^3J(H, H) for protons in positions 1, 2 and 3 are com-patible with the conformational type ^2E. In this conformation the hydrogen atoms of the acetoxy group are very close to the H-14 proton of the oxirane ring (vide supra). Also the carbonyl oxygen of the acetoxy group is in close proximity to the hydroxyl on C-5, their distance being very suitable for an intramolecular hydrogen bond (see discussion of the IR spectra below).

The configuration of the oxirane ring remained so far undetermined since neither the ^{1}H NMR nor the ^{13}C NMR spectra could give convincing arguments. We solved this question using IR-spectroscopic detection of intramolecular hydrogen bonding in arctolide (*Ia*) and the two minor lactones, 3-deacetylarctolide (*II*) and 10,14-de-
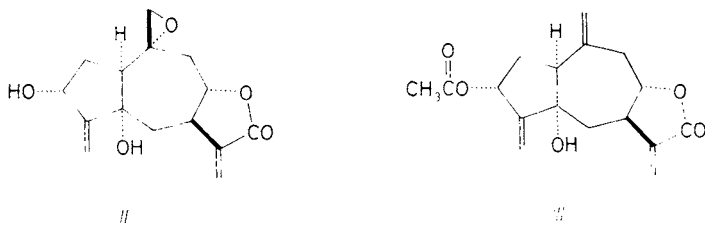
II   *III*

oxoarctolide (*III*) isolated from the same plant material[7] (Table III). The spectrum of 10,14-deoxoarctolide (*III*) exhibits, in addition to the free OH band, a band of bonded hydroxyl corresponding to an intramolecular hydrogen bond to the acetate

carbonyl and confirming the α-configuration of the acetate on C-3 derived from the NMR measurements. Also in the spectrum of 3-deacetylarctolide (*II*), the bands of the free and hydrogen-bonded hydroxyl appear together; because of the distance, the hydroxyl groups on C-3 and C-5 cannot together form a hydrogen bond and the observed chelation is ascribed to a hydrogen bond between the C-5 hydroxyl and the oxirane oxygen atom. The spectrum of arctolide (*Ia*) exhibits two intramolecularly hydrogen-bonded hydroxyl bands, ascribable to hydrogen bonds of the C-5 hydroxyl to the acetate carbonyl and to the oxirane oxygen atom. It is therefore obvious that in arctolide the oxirane ring, as well as the acetoxy and the hydroxy groups, have α-configuration. Thus, using the NMR and IR spectroscopy, the relative configuration of all the asymmetric centers in arctolide, i.e. C-1, C-3, C-5, C-7, C-8 and C-10, has been determined, the configuration at C-3 and C-10 in the previously published[1] structure *I* being now corrected in favour of the formula *Ia*.

However, the structure *Ia* represents only one of the two possible enantiomers. The absolute configuration of arctolide has been established using the CD spectrum and ^{1}H NMR data of 11βH,13-dihydroarctolide (*IVa*), prepared by reduction of the natural compound *Ia* with sodium borohydride. The IR spectrum of *IVa* has proven a γ-lactone ring (1 780 and 1 181 cm^{-1}), an acetate group (1 740 and 1 246 cm^{-1}), a double bond (1 672 cm^{-1}) and a hydroxy group (3 583 and 3 525 cm^{-1}), its mass spectrum contained a molecular ion, m/z 322, and characteristic fragments of m/z 304 (M − 18), 262 (M − 60) and 244 (M − 60 − 18). In the ^{1}H NMR spectrum of *IVa* (Table IV) a doublet of the secondary methyl at δ 1·29 with $J(13, 11)$ 6·5 Hz and a doublet of quartets at δ 2·29 of the H-11 proton appear instead of the exomethylene protons H-13 and H-13' in the spectrum of arctolide. The value of $J(11, 7)$ (12·2 Hz) indicates the *trans*-relation of the protons H-11 and H-7. Upon hydrogenation of the 11,13-double bond, the H-7 signal is shifted upfield (δ 2·45 in *IVa* com-

TABLE III

Hydroxyl absorption bands in the IR spectra of compounds *Ia*, *II* and *III* in carbon tetrachloride
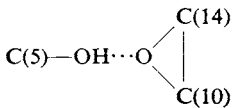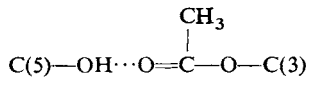
Compound	Free OH	Intramolecularly H-bonded OH	
		C(5)—OH···O⟨C(14)/C(10)	CH$_3$ / C(5)—OH···O=C—O—C(3)
Ia	—	3 551	3 588
II	3 618	3 550	—
III	3 603; 3 618	—	3 540

TABLE IV

Proton NMR parameters of compounds *IVa*, *IVb*, *V*, *VIa* and *VIb* in deuterochloroform

Proton	$\delta(H)$					Protons	$J(H, H)^a$				
	IVa	*IVb*	*V*	*VIa*	*VIb*		*IVa*	*IVb*	*V*	*VIa*	*VIb*
H-1	2·04 bdd	3·02 dd	2·89 dddd	1·65 bdd	3·03 bdd	1, 2	8·0	8·8	9·0	7·8	8·6
H-2	2·49 dt	2·52 dt	2·36 ddd	2·54 dt	2·40—2·60 m	1, 2′	10·1	7·2	3·6	13·7	12·4
H-2′	1·47 ddd	1·46 ddd	2·21 dd	1·40 dt	1·38 ddd	1, 5	—	—	7·5	—	—
H-3	5·67 ddt	5·65 bdd	—	5·29 dt	5·29 dt	2, 2′	14·0	14·4	20·2	13·7	14·0
H-4	—	—	1·91 ddq	2·05 p	3·24 p	2, 3	8·7	8·6	—	8·2	7·9
H-5	—	—	2·25 m	—	—	2′, 3	6·5	6·0	—	4·6	5·9
H-6	2·08 dt	2·88 bd	2·34 ddd	1·99 bd	2·43 bd	3, 15	2·2	2·3	—	0	0
H-6′	1·82 dd	1·83 dd	1·35 dt	1·71 dd	1·76 dd	3, 15′	1·9	2·1	—	0	0
H-7	2·45 ddt	2·24—2·40 m	1·98 m	2·44 bq	2·40—2·60 m	4, 5	—	—	11·9	—	—
H-8	4·17 ddd	4·04 dt	3·98 ddd	4·28 dt	4·21 bq	4, 15	—	—	6·8	7·3	7·4
H-9	2·58 dd	2·29 dd	2·08 dd	2·90 dd	2·70 dd	5, 6	—	—	7·8	—	—
H-9′	1·89 dd	2·10 dd	2·26 ddd	1·59 dd	1·76 dd	5, 6′	—	—	10·4	—	—
H-11	2·29 dq	2·24—2·40 m	2·33 dq	2·29 dq	2·32 dq	6, 6′	15·1	15·5	13·6	14·5	15·4
H-13	1·29 d	1·27 d	1·30 d	1·30 d	1·31 d	6, 7	1·3	1·0	1·0	$\leqq 1$	$\leqq 1$
H-14	2·62 d	2·71 d	2·72 dd	2·65 d	2·61 s	6′, 7	10·4	10·0	10·4	11·2	9·9
H-14′	2·60 d	2·59 d	2·49 dd	2·59 d	2·61 s	7, 8	10·0	10·0	10·0	10·0	9·7
H-15	5·32 d	5·47 d	1·08 d	0·95 d	0·95 d	7, 11	12·2	^{b}	12·0	12·0	12·2
H-15′	5·21 d	5·45 d	—	—	—	8, 9	7·1	5·8	5·2	8·4	7·8
OAc	2·10 s	2·08 s	—	2·08 s	2·09 s	8, 9′	9·1	10·4	11·5	7·8	8·6
NH	—	8·45 s	—	—	8·30 s	9, 9′	14·4	13·2	12·8	15·4	14·9
						11, 13	6·5	6·1	6·9	6·8	6·8
						14, 14′	4·3	4·3	3·7	4·4	^{b}

^{a} Additional long-range couplings observed — in *IVa*, *IVb*: $J(1, 6)$ 0·9; in *V*: $J(1, 14)$ 1·2; $J(2', 4)$ 1·7; $J(9', 14')$ 1·9 Hz; ^{b} the value could not be determined.

pared with δ 3·40 in *Ia*). Neither the chemical shifts of other protons nor coupling constants, nor TAI-acylation shifts in the TAC-derivative *IVb* (Table IV) differ significantly from those for arctolide (*Ia*) and its TAC-derivative *Ib*. The absolute configuration (*R*) at C-11 in 11βH,13-dihydroarctolide (*IVa*) was derived from the negative sign of the CD-maximum at 222 nm ($\Delta\varepsilon$ −1·5), using the sector rule[12,13]. On the basis of the above-discussed relative configurations we assigned 11βH,13--dihydroarctolide the structure *IVa*. This means that the structure *Ia* expresses also the actual absolute configuration of arctolide.
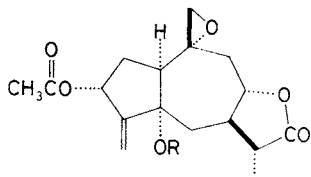
IVa, R = H
IVb, R = CONHCOCCl$_3$
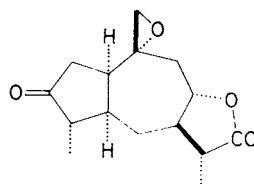
V
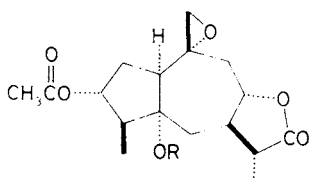
VIa, R = H
VIb, R = CONHCOCCl$_3$

Hydrogenation of arctolide (*Ia*) in ethyl acetate on palladium on carbon afforded, along with some minor products, the tetrahydro derivative *V*, m.p. 156−158°C, $[\alpha]_D^{20}$ +76·3°, of composition $C_{15}H_{20}O_4$ (*M* 264). Its IR spectrum proved the presence of a γ-lactone grouping (1 778 and 1 183 cm^{-1}) and a ketone in a five--membered ring (1 740 cm^{-1}). In the CD spectrum we observed a positive maximum at 295 nm ($\Delta\varepsilon$ +2·7) and a negative one at 216 nm ($\Delta\varepsilon$ −1·3). The long-wavelength maximum confirmed the presence of a carbonyl group; so did the ORD measurements which found a maximum at 311 nm ($[\Phi]$ + 5 960) and at 272 nm ($[\Phi]$ − 7 220). Proton NMR spectrum (Table IV) confirmed the reduction of both the exomethylene groups under formation of secondary methyl groups (doublets at δ 1·30 and 1·08 with *J* 6·3 and 6·8 Hz). The acetate on C-3 was replaced by carbonyl and the hydroxyl on C-5 by hydrogen (multiplet at δ 2·25). The value of *J*(7, 11) (12·0 Hz) again indicates the *trans*-relation of the H-7 and H-11 protons as in *IIa*.

The coupling constant $J(1, 5)$ $(7 \cdot 5 \text{ Hz})$ is in accord with the *cis*-arrangement of the H-1 and H-5 protons and hence the *cis*-annelation of the five- and the seven-membered homocycles, analogously to *Ia*. The high value of $J(4, 5)$ $(11 \cdot 9 \text{ Hz})$ proves the antiperiplanar arrangement of the H-4 and H-5 protons and the α-orientation of the C-4 methyl group. Comparison of the $^3J(\text{H, H})$ values in compounds *V* and *Ia* shows that introduction of carbonyl into the position 3 and hydrogenation of the C-4 exomethylene induces only a conformational change of the five-membered homocycle. The absolute configuration of compound *V*, derived from the CD data, agrees with the suggested absolute configuration *Ia* for arctolide. Obviously, the keto lactone *V* has arisen by a combination of a garryfolin–cuauchichin rearrangement (see e.g. refs[14−17]) accompanied with deacetylation under formation of carbonyl on C-3, and hydrogenolysis of the hydroxyl on C-5.

The hydrogenation of arctolide *(Ia)* afforded further the amorphous 4αH,11βH--tetrahydroarctolide *(VIa)* of composition $C_{17}H_{24}O_6$ and $[\alpha]_D^{20}$ $+10 \cdot 7°$. According to IR spectrum, the compound *VIa* contained a γ-lactone grouping $(1\ 775$ an $1\ 181 \text{ cm}^{-1})$ and an acetoxy group $(1\ 735$ and $1\ 248 \text{ cm}^{-1})$. Mass spectrum showed no molecular ion but contained a characteristic fragment of m/z 264 $(\text{M} - 60)$. CD spectrum exhibited a maximum at 214 nm $(\Delta\varepsilon \ -1 \cdot 5)$. The $^1\text{H NMR}$ spectrum (Table IV) displayed signals of two secondary methyl groups (doublets at δ 0·95 and 1·30, J 7·3 and 6·8 Hz) instead of the exomethylene proton signals. This behaviour is similar as in the case of compound *V*; however, compound *VIa* contains both an acetate (singlet at δ 2·08) and a hydroxyl group. The value of $J(11, 7)$ $(12 \cdot 0 \text{ Hz})$ again indicates *trans*-relation between the H-11 and H-7 protons and the α-configuration of the methyl group on C-11. The presence of hydroxyl on C-5 was proven by in situ TAI-acylation under formation of the TAC-derivative *VIb*. The large TAI-acylation shifts of the H-1 and H-4 proton signals (1·38 and 1·19 ppm, respectively), together with no effect on the C-4 methyl group, has shown the *cis*-orientation of hydroxyl relative to the H-1 and H-4 hydrogen atoms and consequently the β-configuration of the C-4 methyl. The absolute configuration of 4αH,11βH-tetrahydroarctolide *(VIa)* was derived, as for the keto lactone *V*, from the negative sign of the CD maximum at 214 nm by application of the sector rule[12,13] and agrees with the absolute configuration of arctolide *Ia*.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Silica gel for column chromatography (30—60 μm) was deactivated by addition of 11% of water. Thin-layer chromatography was carried out on silica gel G (Merck). IR spectra were recorded in chloroform, unless stated otherwise, on a Perkin–Elmer PE 580 spectrophotometer. Mass spectra were measured on an AEI MS 902 spectrometer, optical rotations were determined in methanol on a Perkin--Elmer 141 polarimeter. CD spectra were obtained with a Roussel–Jouan CD 185 dichrographe in methanol, ORD spectra on a spectrophotometer JASCO ORD/UV5 in the same solvent.

Proton and ^{13}C NMR spectra (at 200 and 50·3 MHz, respectively) were taken on an FT-NMR spectrometer Varian XL-200 in deuterochloroform at about 22°C with tetramethylsilane as internal standard. All the measurements were performed in a 5 mm tube (for ^{1}H NMR: about 15 mg of sample in 0·5 ml of solution, for ^{13}C NMR: about 150 mg in 0·5 ml). In all the NMR experiments we used pulse sequences that are part of the standard software (version H1.Z) of the spectrometer. Chemical shifts and coupling constants of protons were obtained by the first order analysis from the expanded spectra (2 Hz/cm), measured with digital resolution of 0·2 Hz, using the Gaussian transformation of FID (ref.[18]) to enhance resolution.The TAC-derivatives *Ib*, *IVb* and *VIb* were prepared in situ by adding trichloroacetyl isocyanate[3] (slight excess) directly into a solution of *Ia*, *IVa* or *VIa* in an NMR tube.

For the homonuclear ^{1}H-*J*-resolved 2D-NMR spectrum (Fig. 2) the pulse sequence HOM2DJ (ref.[19]) was used, the parameters being as follows: spectral width 1 100 Hz (δ(H)) and 50 Hz (*J*(H, H)), acquisition time 0·466 s, pulse width 8 µs (flip angle 90°), 4 transients for each of 256 increments in the evolution period, matrix 1 024 $\times$ 512 data points, total measurement time 2 h.

For the homocorrelated ^{1}H–^{1}H 2D-NMR spectrum (Fig. 3) the pulse sequence HOMCOR (ref.[20]) was used. Parameters: spectral width 1 100 Hz in both dimensions, acquisition time 0·233 s, pulse width 5 µs (flip angle 55°), 4 transients for each of 256 increments, matrix 512 $\times$ 512 data points, total measurement time 1 h.

For homonuclear ^{1}H–^{1}H NOE 2D-NMR spectrum (Fig. 4) we employed pulse sequence NOE2D (refs[21,22]) with the same principal parameters as in the preceding HOMCOR experiment. For magnetization transfer the mixing time was 0·1 s and equilibration delay was 3·767 s. Total measurement time 2·5 h.

Proton-decoupled ^{13}C NMR spectrum (Fig. 5) was taken with broad-band proton decoupling switched on during the whole measurement, the decoupling frequency being in the middle of the proton spectrum. The parameters were as follows: spectral width 10 000 Hz, acquisition time 1 s, pulse width 5 µs (flip angle 50°), equilibration delay 1 s, number of transients 400, line--broadening factor 1 Hz, measurement time 13 min.

Off-resonance proton-decoupled ^{13}C NMR spectrum (Fig. 6) was measured under analogous conditions as the preceding one except that the continuous-wave proton decoupling had the frequency placed 1 500 Hz upfield from the middle of the ^{1}H NMR spectrum and the line--broadening factor was 3 Hz.

For the "attached proton test" ^{13}C NMR spectrum (Fig. 7) the APT pulse sequence[23] was used, the parameters being the same as given for Fig. 5 and the *J*-modulation lasting 7 ms (corresponds to ^1J(C, H) 143 Hz).

The edited proton-decoupled ^{13}C NMR spectra (Fig. 8) were obtained using the DEPT pulse sequence[24]. The four acquired DEPT spectra (with the ^{1}H pulse values Θ 45, 90, 90 and 135°) were then automatically edited using the ADEPT program. Parameters used: spectral width 10 000 Hz, acquisition time 0·8 s, 16 K data points, pulse width 8·5 µs (flip angle 90°), equilibration delay 5 s, proton pulse 50 µs (90°) with a decoupler, saturation time 0·05 s (corresponds to average value ^1J(C, H) 135 Hz), 80 transients for each of four spectra, line broadening factor 3·5 Hz, total measurement time c. 40 min.

The relaxation times T_1 of ^{13}C carbon atoms were measured by the "inversion-recovery" technique using the AUTOT1 pulse sequence (Fig. 9). Parameters: spectral width 10 000 Hz, equilibration delay 20 s, 180° pulse (17 µs), variable relaxation delays: 0·01, 0·03, 0·1, 0·3, 1·0, 2·5, 7·0, 20·0 and 60 s, observe pulse 8·5 µs (90°), acquisition time 0·8 s, 16 K data points, 8 transients for each of 9 relaxation delays, proton decoupler "on" during all the measurement time, line broadening factor 3·5 Hz, measurement time c. 40 min. The relaxation times were calculated using a program which is a part of the AUTOT1 pulse sequence.

Heteronuclear ^{13}C–^1H correlated 2D-NMR spectrum (Fig. 10) was obtained using a HETCOR pulse sequence[25]. Parameters: spectral width 10 000 Hz (^{13}C) and 2 000 Hz (^1H), acquisition time 0·102 s, observe ^{13}C pulse 8·5 μs (flip angle 90°), proton pulse 50 μs (90°) with decoupler, equilibration delay 2 s, the used average value of $^1J(C, H)$ (135 Hz) gives the delays D3 = 3·7 ms and D4 = 2·5 ms, delay values in evolution period were incremented with 64 increments, 200 transients were accumulated for each of the 64 values, matrix 2 048 × 512 data points (zero filling from 128 real points to 512 for resolution enhancement in ^1H dimension), measurement time 7·5 h.

Heteronuclear ^{13}C–^1H 2D-J-resolved spectrum (Fig. 11) was measured using the HET2DJ pulse sequence[26] in the "gated-decoupler" version. Parameters: spectral width 8 400 Hz ($\delta(^{13}C)$) and 400 Hz ($J(C, H)$), observe ^{13}C-pulse 8·5 μs (flip angle 90°), acquisition time 0·122 s, equilibration delay 5 s, 80 transients accumulated for each of 128 increments, matrix 2 048 × 256 data points, total measurement time 15·5 h.

Proton-coupled ^{13}C NMR spectrum with NOE enhancement (Fig. 12) was measured with the following parameters: spectral width 8 000 Hz, pulse width 8·5 μs (flip angle 90°), acquisition time 2 s, 32 K data points, broadband proton-decoupling switched "on"during 2 s before acquisition and switched "off" during the data acquisition, 9 000 transients accumulated, measurement time 10 h.

11βH,13-Dihydroarctolide (*IVa*)

A suspension of sodium borohydride (2 g) in methanol was added dropwise at room temperature to a stirred solution of arctolide (*Ia*; 1·0 g) in methanol (10 ml). After stirring for 20 min, the mixture was decomposed with water, acidified with 2% sulfuric acid to pH 4 and methanol was distilled off under reduced pressure. The residue was extracted with chloroform, the combined chloroform extracts were washed with water to neutrality, dried over anhydrous sodium sulfate and the solvent was evaporated. Chromatography of the residue afforded 11βH,13-dihydro-arctolide (*IVa*; 435 mg), m.p. 151—152°C, $[\alpha]_D^{20}$ +89·1° (*c* 0·354). For $C_{17}H_{22}O_6$ (322·3) calculated: 63·34% C, 6·88% H, 0·31% H act.; found: 63·65% C, 6·80% H, 0·34% H act. Mass spectrum, m/z: 322 (M), 304 (M — 18), 262 (M — 60), 244 (M — 60 — 18). IR spectrum (cm^{-1}): 1 780, 1 181 (γ-lactone), 1 740, 1 246 (acetate), 1 672 (C=C), 3 585, 3 525 (OH). CD spectrum, nm ($\Delta\varepsilon$): 222 (− 1·5).

Lactone *V* and 4αH,11βH-Tetrahydroarctolide (*VIa*)

A solution of arctolide (*Ia*; 640 mg) in ethyl acetate (25 ml) was hydrogenated on a Pd/C catalyst (100 mg) at room temperature and atmospheric pressure until the hydrogen absorption ceased (about 6 h). The catalyst was filtered off, ethyl acetate evaporated under diminished pressure and the residue (560 mg) chromatographed on a column of silica gel (50 g). The first fractions afforded the lactone *V*, m.p. 156—158°C, $[\alpha]_D^{20}$ +76·3° (*c* 0·333). For $C_{15}H_{20}O_4$ (264·3) calculated: 68·16% C, 7·73% H; found: 68·04% C, 7·36% H. Mass spectrum, m/z: 264 (M). IR spectrum (cm^{-1}): 1 778, 1 183 (γ-lactone), 1 740 (C=O in 5-membered ring). CD spectrum, nm (ΔE): 295 (+2·7), 216 (−1·3). ORD, nm (Φ): 311 (+5 960); 296 (±0); 272 (−7 220); $a = +132$. Further fractions afforded non-crystalline 4αH,11βH-tetrahydroarctolide (*VIa*), $[\alpha]_D^{20}$ +10·7° (*c* 0·277). For $C_{17}H_{24}O_6$ (324·3) calculated: 62·96% C, 7·46% H; found: 62·68% C, 7·23% H. Mass spectrum, m/z: 264 (M — 60). IR spectrum (cm^{-1}): 1 775, 1 181 (γ-lactone), 1 735, 1 248 (acetate), 3 530 (OH). CD spectrum, nm ($\Delta\varepsilon$): 214 (−1·5).

and interpreted by Dr L. Dolejš. Optical rotations were determined by Mrs Z. Ledvinová. We express our thanks to all of them.

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Translated by M. Tichý.