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Structures of Metasequoic Acid A and B

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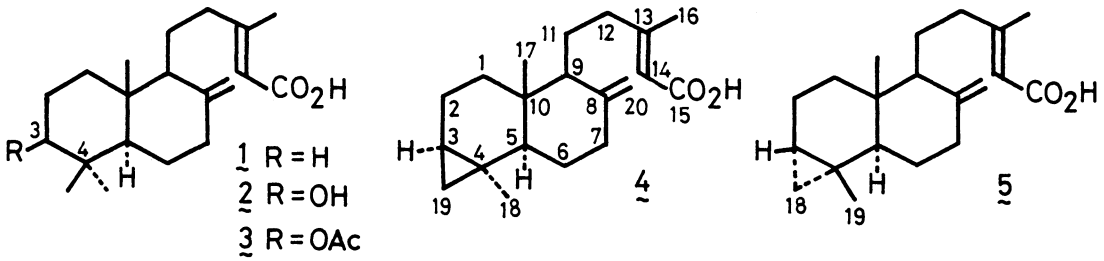
Two new antifungal diterpenes, metasequoic acid A(4) and B(5) were isolated from Metasequoia glyptostroboides Hu et Cheng. These compounds possess a new skeleton which contains a cyclopropane ring bridging C-3 and C-4 of the labdane framework. The structures 4 and 5 have been determined by spectroscopic methods.

Labdane type diterpenes, 1,[1] 2, and 3, which showed the antifungal activity against Pyricularia oryzae, were previously isolated from Metasequoia glyptostroboides Hu et Cheng in our laboratory.[2] In the following, we describe the structure elucidation of two new skeletal diterpenes, metasequoic acid A(4) and B(5) isolated as minor constituents from the same source. These compounds were also found to inhibit the spore germination of P. oryzae with almost the same strength as that of 1.[2b]



The twigs of the plant were extracted with hexane and the extracts were fractionated to give acidic chloroform-soluble oil. Compound 4($C_{20}H_{30}O_2$,[3] mp 112-113 °C, $[\alpha]_D$ +36.3°) and 5($C_{20}H_{30}O_2$,[3] mp 124-125 °C, $[\alpha]_D$ +94.0°) were isolated

Table 1. NMR data of metasequoic acid A(4) and B(5)[a]

	Compd. 4		Compd. 5			Compd. 4			Compd. 5		
C	δ	mult.[b]	δ	mult.[b]	H	δ	mult.	J/Hz	δ	mult.	J/Hz
1	34.7	t	32.9	t	1α	0.99[c]	ddd	14,9,5	0.74	ddd	13,13,6
					1β	1.54[c]			1.63	ddd	13,6,2
2	21.0	t	20.0	t	2α	2.01[c]			1.76	ddd	15,6,2
					2β	1.54[c]			1.93[c]		
3	18.0	d	19.2	d	3	0.65	ddd	9,9,5	0.59	ddd	9,6,6
4	19.6	s	6.5	s							
5	50.2	d	51.4	d	5α	1.54[c]			1.25	dd	13,4
6	25.9	t	28.3	t	6α	1.90	dddd	13,6,3,3	1.89[c]		
					6β	1.28	dddd	13,13,13,5	1.44[c]		
7	38.1	t	38.3	t	7α	2.08	ddd	13,13,6	1.93[c]		
					7β	2.44	ddd	13,5,3	2.39	ddd	13,5,3
8	148.4	s	148.2	s							
9	55.6	d	52.5	d	9α	1.60[c]			1.46[c]		
10	38.7	s	38.7	s							
11	21.8	t	22.4	t	11	1.48[c]			1.48[c]		
						1.66[c]			1.68[c]		
12	39.8	t	40.2	t	12	1.99[c]			1.99[c]		
						2.31[c]			2.32[c]		
13	161.0	s	163.8	s							
14	114.9	d	114.9	d	14	5.68	bs		5.67	bs	
15	167.3	s	172.2	s							
16	18.9	q	19.2	q	16	2.16	d	2	2.16	d	2
17	13.1	q	11.6	q	17	0.51	s		0.66	s	
18	28.2	q	21.7	t	18(Me)	1.02	s				
					(exo)				0.43	dd	9,4
					(endo)				0.05	dd	6,4
19	18.0	t	24.0	q	19(Me)				0.93	s	
					(exo)	0.19	dd	9,5			
					(endo)	0.45	dd	6,4			
20	107.3	t	106.9	t	20	4.53	bs		4.53	bs	
						4.88	bs		4.89	bs	

a) Both spectra(^{1}H NMR; at 360 MHz, ^{13}C NMR; at 25 MHz) were measured in $CDCl_3$. b) Multiplicities were determined by DEPT method. c) Resonances were not clearly separated and δ values were read from ^{1}H-^{1}H and ^{13}C-^{1}H 2D maps.

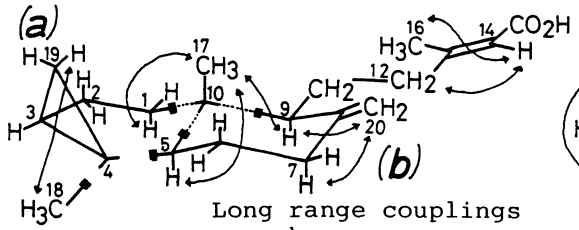
from the acidic oil by layer chromatography(SiO_2, ether/benzene) followed by prep. HPLC(C_{18}-reverse phase, AcOH/H_2O/MeOH/MeCN).

The usual IR, ^{1}H NMR(360 MHz), and ^{13}C NMR(25 or 90 MHz) spectroscopy indicated that each compound has a exo-methylene group(4: ν_{max} 890 cm^{-1}; δ4.53, 4.88; δ107.3, 148.4, 5: ν_{max} 900 cm^{-1}; δ4.53, 4,89; δ106.9, 148.2),[2] a moiety of $-C(CH_3)=CHCO_2H$(4: ν_{max} 2800, 1690, 1645 cm^{-1}; δ2.16, 5.68; δ18.9, 114.9, 161.0, 167.3, 5: ν_{max} 2800, 1685, 1640 cm^{-1}; δ2.16, 5.67; δ19.2, 114.9, 163.8, 172.2),[2] two quaternary methyl groups(4: δ0.51, 1.02, 5: δ0.66, 0.93), and a cyclopropane ring(4: δ0.19, 0.45, 0.65, 5: δ0.05, 0.43, 0.59). In considering these results and molecular formulas,[3] both 4 and 5 were assumed to be tricyclic diterpenes closely related to the compound 1.

The complete ^{1}H NMR assignments of 4 and 5 were obtained by ^{1}H-^{1}H COSY and

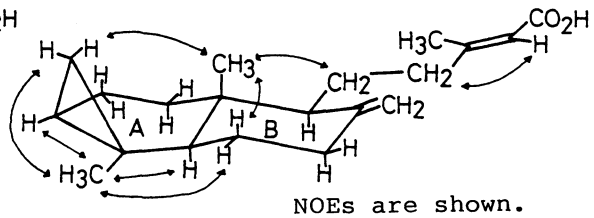
extensive NOE measurements. On the basis of these assignments, both of ^{13}C NMR were analyzed by ^{13}C-^{1}H correlation method.[4] The results are shown in Table 1.

Analysis of the ^{1}H-^{1}H correlation spectrum of metasequoic acid A(4) indicated that two continuous proton systems, (a) and (b) were disclosed as depicted in 4a. Long range couplings observed in the COSY spectrum are also shown in 4a. The resonances of C(1)-Hβ, C(2)-Hβ, C(5)-H, C(9)-H, and C(11)-H_2 were closely observed at about δ1.5. The analysis of these overlapped signals was greatly facilitated by ^{13}C-^{1}H COSY spectrum.



Long range couplings are shown.

4a

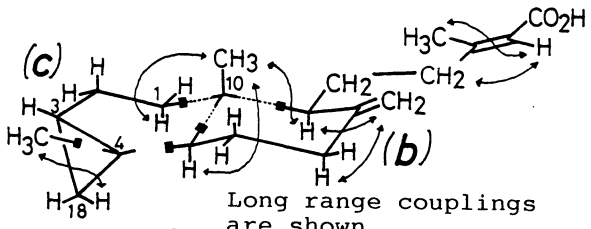


NOEs are shown.

4b

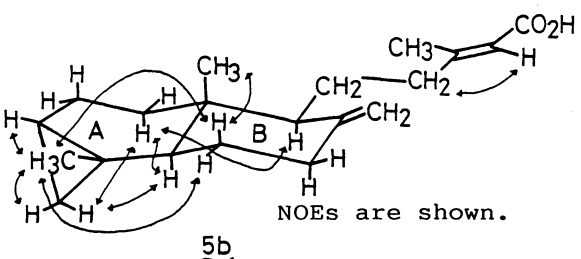
Careful inspection of the homonuclear 2D map of 4 revealed the occurrence of cross peaks due to W-type long range couplings of C(17) methyl protons with C(1)-, C(5)-, and C(9)-Hα. These observation connected the two fragments at C(10) as shown in 4a with doted lines and suggested the stereochemistry at C(5), C(9), and C(10).

The results of NOE experiment are shown in 4b. The NOE observed between C(17) methyl protons and C(19)-H_{endo} indicated the β-orientation of cyclopropane ring. Thus the structure of metasequoic acid A(4) was determined as to be consistent with that shown in 4b. The other NOEs also supported the assigned structure.



Long range couplings are shown.

5a



NOEs are shown.

5b

The COSY spectrum of metasequoic acid B(5) disclosed the occurrence of two partial proton systems, (b) and (c) as shown in 5a. The whole proton system was assigned in taking account of W-type long range couplings (shown in 5a). The α-orientation of cyclopropane ring was confirmed by the observation of NOE between C(1)-Hα and C(18)-H_{endo}. Thus the structure of metasequoic acid B(5) was determined as to be 5b, the diastereoisomer of metasequoic acid A(4) with respect to the

orientation of cyclopropane ring.

The coupling data of 4 and 5 suggested the chair conformation of both B rings. The lack of cross peaks due to the couplings of C(3)-H/C(2)-Hβ(in 4) and C(3)-H/C(2)-Hα(in 5) in the 2D maps indicated the dihedral angles of H-C(3)-C(2)-Hβ(in 4) and H-C(3)-C(2)-Hα(in 5) to be approximately 90°. In considering these results, the A ring of each compound was assumed to be flattened from the chair form as might be expected from the fusion of cyclopropane at C(3) and C(4).

The structure of metasequoic acids represented a modified labdane skeleton with a cyclopropane ring bridging C(3) and C(4). The presence of cyclopropane ring in labdane is not known so far. Several modified pimarane diterpenes containing a cyclopropane ring fused at C(3) and C(4) were isolated from marine red alga and sea hare.[5)]

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