Bruceines D, E and H

Xian Li* and Lijun Wu

Shenyang College of Pharmacy
Shenyang, China

Yaeko Konda, Mieko Iguchi, Hiroshi Takahasi,
Yoshihiro Harigaya* and Masayuki Onda

School of Pharmaceutical Sciences, Kitasato University,
Tokyo 108, Japan

Received August 30, 1988

The absolute stereochemistry of bruceines D, E and H has been confirmed to be 1*S*, 5*S*, 7*R*, 8*R*, 9*R*, 10*S*, 11*R*, 12*S*, 13*R*, 14*R*, 15*R* and additionally 2*S* for bruceine E by a combination of 2D nmr [^{1}H-^{1}H and ^{1}H-^{13}C (one-bond and long-range) COSY] nOe and cd spectroscopic analyses.

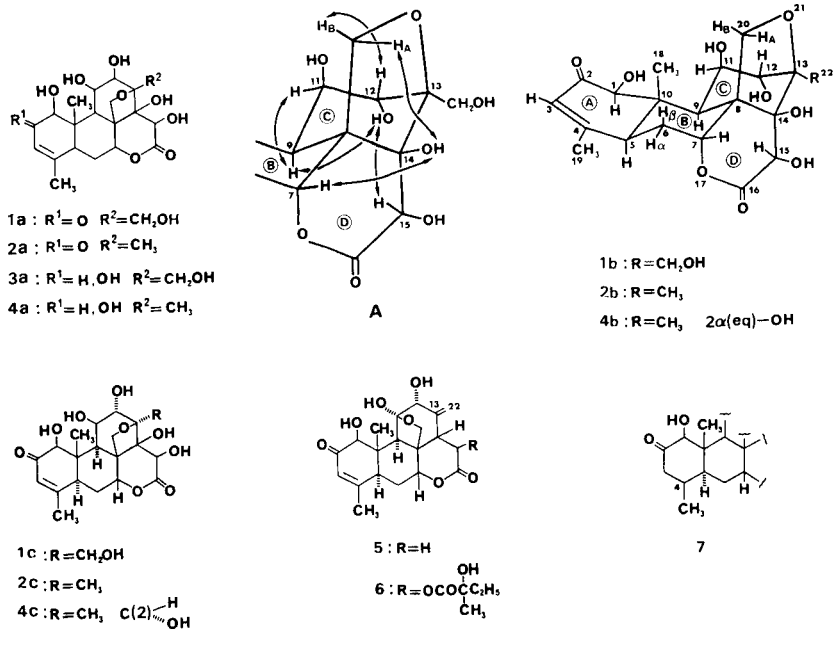
J. Heterocyclic Chem., **26**, 493 (1989).

A number of quassinoids containing a 13,20-epoxy function have so far been isolated from the family Simaroubaceae [1-6]. Recently, in the course of the studies on the bitter principles of this family, a new quassinoid was isolated along with bruceines B, D, E and F and brusatol from *Brucea javanica* by us (Chinese group) [7]. It was deduced that this substance was 22-hydroxybruceine D named bruceine H (**1**) by the comparison with the spectroscopic properties of bruceine D (**2**). Takahashi *et al.* [5] isolated yadanziolides A, B and C from the same plant. It was stated that yadanziolide A was identical with the same ketone as **1**, which was obtained by the oxidation of bruceine F (**3**). Polonsky *et al.* [2,6] and Lee *et al.* [3] already reported the structures of **2** and **3** by means of ^{1}H nmr (60 and 100 MHz) spectroscopy. Since then, it has been considered that their absolute stereochemistry was decided [8]. However, in our opinion, their absolute stereochemistry was not convincingly demonstrated. In particular, the 14-OH and 15-OH configurations were only deduced from the biosynthesis of other quassinoid groups [8]. This paper is concerned with an approach to the absolute stereochemistry of **1** and **2** together with bruceine E (**4**) [2,4] by a combination of high-resolution (^{1}H, 400 MHz; ^{13}C, 100.6 MHz) nmr and cd spectroscopic analyses.

NMR Spectroscopy.

The nmr spectra were taken in pyridine-d_5 unless otherwise noted. The 1D nmr spectra of **1**, **2**, and **4** showed the number of hydrogens and carbons corresponding to each molecular formula. The number of hydrogens attached to each individual carbon was determined by DEPT experiments. Hydroxyls were examined in DMSO-d_6 solution

Scheme 1



and were exchangeable with deuterium oxide. The unambiguous assignments of protons and carbons were straightly made by ^{1}H-^{1}H and ^{1}H-^{13}C COSY techniques, which were particularly effective to established each of overlapping protons observed for **4**, being in accord with the gross structures **1a**, **2a** and **4a** shown in Scheme 1. The 2D nmr spectra of these quassinoids are published for the first time. It was found that the assignments of 1-H and 12-H in **1** (yadanziolide A) and **2** made by Takahashi *et al.* [5] must be reversed and that the ^{1}H and ^{13}C nmr spectra of **2** reported by Lee *et al.* [3] should be reappraised. The relative stereochemistry was established by nOe difference spectroscopy and ^{1}H-^{1}H decoupling experiments.

Bruceine H (**1**).

The nmr spectra of **1** are shown in Figures 1-4. The spectral parameters including ^{1}H-^{1}H coupling constants are given in Table I. Each proton and carbon belonging to methyls, methylenes and methines were readily shown to correspond to each other by the ^{1}H-^{13}C (one-bond) COSY spectrum. Thus, the assignments of these carbons were straightforward from those of protons which were made as follows.

Two methyls, 18-H_3 (angular) and 19-H_3 (vinylic), were easily distinguished from each other by the ^{1}H-^{1}H and ^{1}H-^{13}C (long-range) COSY spectra. The empirical assignments of two methyls would be possible by the comparison of the proton and carbon chemical shifts with those of related quassinoids. ^{1}H-^{1}H decoupling experiments provided the presence of the C(5)H-C(6)H_2-C(7)H moiety. The distinction between 5-H and 7-H was based on the ^{1}H-^{1}H

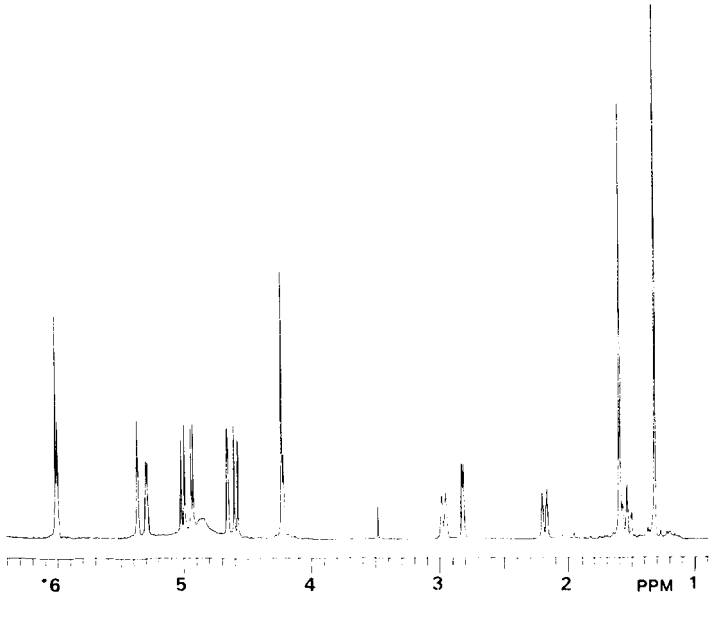


Figure 1. ^{1}H Nmr spectrum of Bruceine H (1).

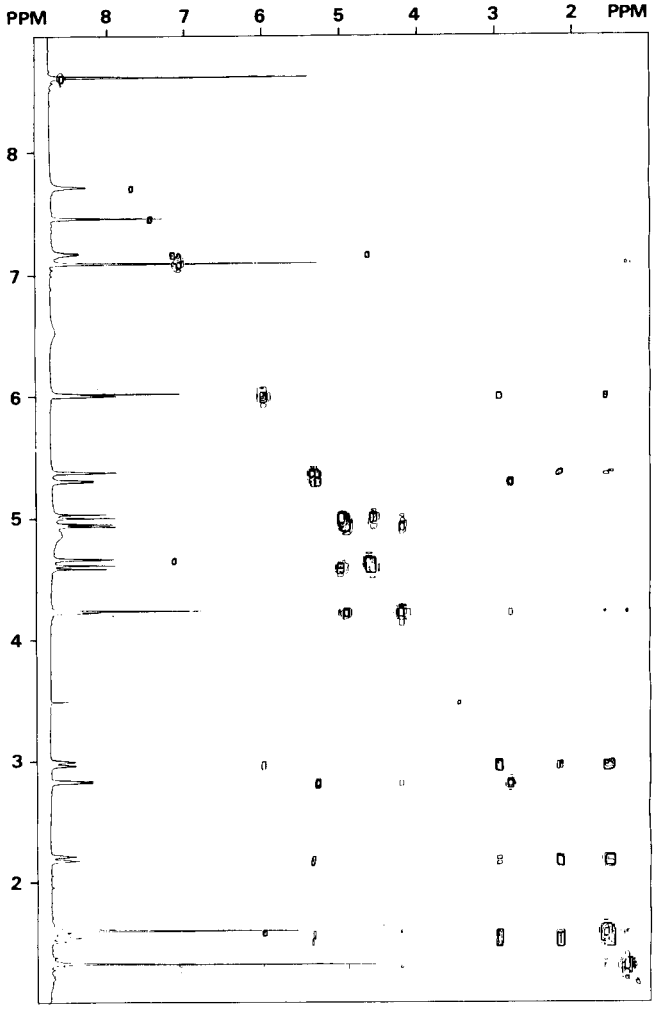


Figure 2. ^{1}H-^{1}H COSY spectrum of Bruceine (1).

COSY spectrum showing couplings between 3-H (vinylic) and 5-H and between 7-H and 20-H_A. Two methylenes, 20-H_2 and 22-H_2, were easily identified (^{1}H-^{1}H and ^{1}H-^{13}C). The ^{1}H-^{1}H decoupling experiments showed that 11-H was vicinally coupled to 9-H which was correlated to 20-H_A (^{1}H-^{1}H), and 1-H, 7-H, 12-H and 18-H_3 (^{1}H-^{13}C). Two methines, 1-H and 12-H, were unambiguously assigned: 1-H was correlated to 18-H_3 (^{1}H-^{1}H and ^{1}H-^{13}C); 12-H was associated with 11-H (^{1}H-^{1}H and ^{1}H-^{13}C) and 22-H_A (^{1}H-^{13}C). Two methines, 3-H (vinylic) and 15-H, were readily identified (^{1}H-^{1}H and ^{1}H-^{13}C). Assignments of five hydroxyls were smoothly made by the ^{1}H-^{1}H COSY spectrum taken in DMSO-d_6. The orientation of each proton belonging to 6-H_2 and 20-H_2 was confirmed in the course of nOe experiments. Quaternary carbons were unambiguously assigned by the ^{1}H-^{13}C (long-range) COSY spectrum.

The presence of a 13,20-epoxy function sterically re-

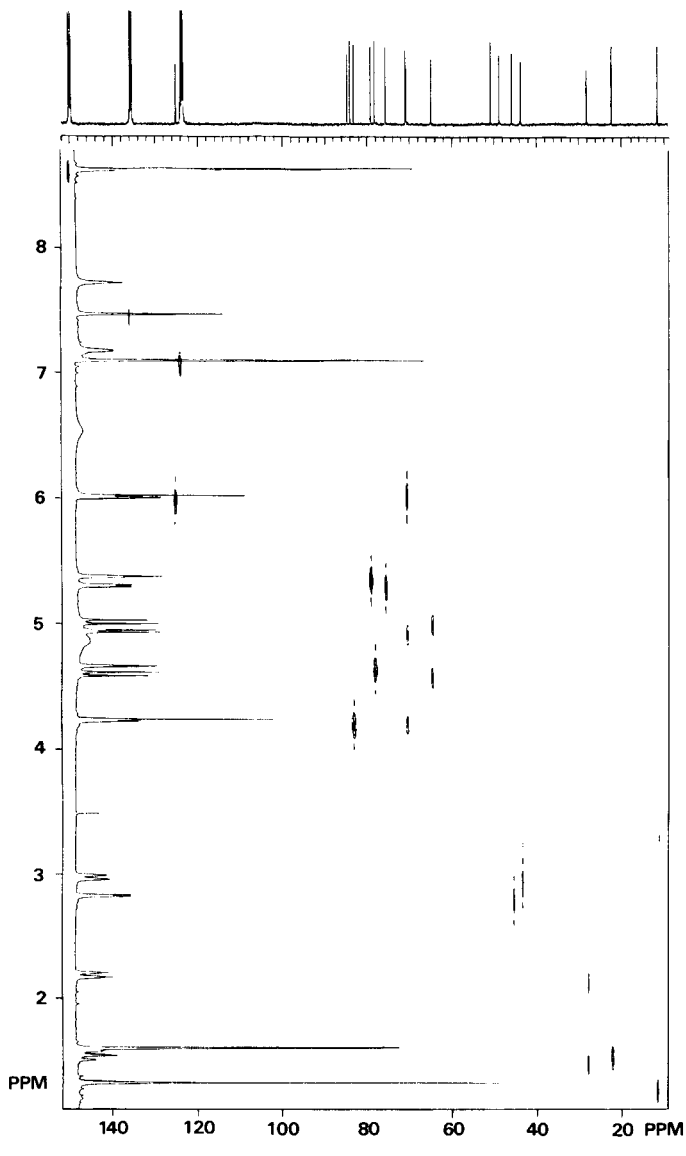


Figure 3. ^{1}H–^{13}C (one-bond) COSY spectrum of Bruceine H(1).

Table I

NMR Data for Bruceine H (1) [a]

No	Carbon δ c	Correlated proton [b] One-bond [d]	Long-range	^{1}H-^{1}H [c]
1	83.255 d	1-H 4.228 s (4.272 d)	18-H_3	18-H_3 (1-OH)
2	198.693 s		1-H, 9-H	
3	125.130 d	3-H 5.999 m	5-H, 19-H_3	5-H, 19-H_3
4	163.582 s		5-H, 19-H_3	
5	43.750 d	5-H 2.750 br d (2.905 br d)	3-H, 7-H, 18-H_3, 19-H_3	3-H, 6α-H, 6β-H (19-H_3)
6	28.129 t	6α-H 2.187 dt 6β-H 1.541 ddd		5-H, 6β-H 7-H 5-H, 6α-H 7-H
7	79.261	7-H 5.368 t		6α-H, 6β-H 20-H_A
8	50.854 s		6α-H, 7-H 11-H	
9	45.844 d	9-H 2.822 d (2.338 d)	1-H, 7-H 12-H, 18-H_3	11-H, 20-H_A
10	48.805 s		1-H, 6α-H, 9-H, 18-H_3	
11	75.732 d	11-H 5.293 d (4.375 d)	12-H	9-H, 12-H (11-OH)
12	78.343 d	12-H 4.653 s (3.703)	11-H, 22-H_A	11-H, 12-OH
13	84.808 s		12-H, 20-H_B 22-H_A, 22-H_B	
14	84.096 s		12-H, 20-H_B 22-H_A, 22-H_B	
15	70.939 d	15-H 6.015 s (4.958 d)		(15-OH)
16	175.348 s		15-H	
18	11.653 q	18-H_3 1.323 s	1-H, 9-H	1-H
19	22.347 q	19-H_3 1.600 s (1.885 s)	3-H	3-H (5-H)
20	70.795 t	20-H_A 4.227 d (3.703) 20-H_B 4.933 d	7-H, 9-H	7-H, 9-H 20-H_B 20-H_A
22	64.952 t	22-H_A 4.590 d (3.703) 22-H_B 5.007 d (3.938 dd)	12-H	22-H_B (22-OH) 15-H, 22-H_A (22-OH)
		1-OH (5.298 d)		(1-H)
		11-OH (3.703) (overlapped with 20-H_A)		(11-H)
		12-OH 7.168 s (5.257 d)		12-H
		14-OH (5.900 s)		
		15-OH (5.463 d)		(15-H)
		22-OH (5.095 t)		(22-H_A, 22-H_B)

[a] The spectra were taken on a Varian XL-400 (^{1}H, 400 MHz, ^{13}C, 100.6 MHz) in pyridine-d_5, ppm. [b] These data were obtained by ^{1}H-^{13}C COSY experiments. [c] These data were obtained by ^{1}H-^{1}H COSY experiments. The data in parentheses were additionally obtained in DMSO-d_6. Coupling constants (Hz) were as follows: $J_{5,6\alpha}$ = 2.3, $J_{5,6\beta}$ = 12.7, $J_{6\alpha,\beta}$ = 14.3, $J_{6\alpha,7}$ = $J_{6\beta,7}$ = 2.3, $J_{9,11}$ = 4.5, $J_{20A,B}$ = 6.7, $J_{22A,B}$ = 11.5 ($J_{1,1\text{-OH}}$ = 3.5, $J_{12,12\text{-OH}}$ = 4.5, $J_{15,15\text{-OH}}$ = 5.5, $J_{22A,B,22\text{-OH}}$ = 6.0). [d] The data in parentheses were obtained in DMSO-d_6.

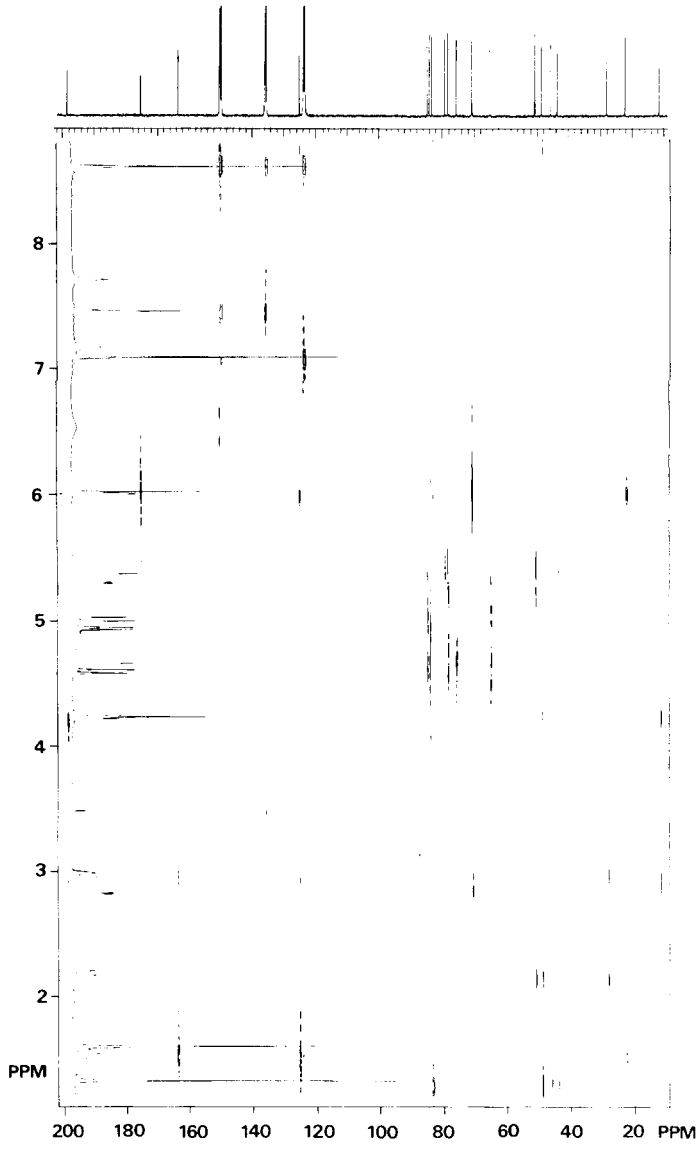


Figure 4. ^{1}H-^{13}C (long-range) COSY spectrum of Bruceine H(1).

Table II

NMR Data for Bruceine D (**2**) [a]

No	Carbon δ c	Correlated proton [b] One-bond [d]	Long-range	^{1}H-^{1}H [c]
1	83.329 d	1-H 4.200 s (4.260 d)	3-H, 18-H_3	18-H_3 (1-OH)
2	198.709 s		1-H, 5-H 19-H_3	
3	125.088 d	3-H 5.991 s	5-H, 19-H_3	5-H, 19-H_3
4	163.650 s		5-H, 19-H_3	
5	43.660 d	5-H 2.948 br d (2.889 br s)	3-H, 6β-H 7-H, 18-H_3 19-H_3	3-H, 6α-H, 6β-H (19-H_3)
6	28.144 t	6α-H 2.173 dt 6β-H 1.527 ddd	5-H	5-H, 6β-H 7-H 5-H, 6α-H 7-H
7	79.671 d	7-H 5.392 t (4.907 t)		6α-H, 6β-H
8	50.409 s		6α-H, 9-H *11-H, 20-H_A*	
9	45.968 d	9-H 2.783 d	1-H, 7-H 12-H, 18-H_3	11-H, 20-H_A
10	48.852 s		1-H, 5-H, 6α-H, 9-H 18-H_3	
11	75.764 d	11-H 5.309 d (4.375 t)	9-H, 12-H	9-H, 12-H (11-OH)
12	81.776 d	12-H 4.472 s (3.526 d)	11-H, 22-H_3	11-H, 12-OH
13	84.909 s		11-H, 12-H 22-H_3	
14	82.643 s		12-H, 15-H 22-H_3	
15	70.629 d	15-H 5.991 s (4.953 d)		(15-OH)
16	175.406 s		15-H	
18	11.682 q	18-H_3 1.323 s	1-H, 9-H	1-H
19	22.337 q	19-H_3 1.578 s (1.882 s)	3-H	3-H (5-H)
20	70.079 t	20-H_A 4.746 d 20-H_B 4.846 d	7-H, 9-H	9-H, 20-H_B 20-H_A
22	19.672 q	22-H_3 1.963 s	12-H	
		1-OH (5.287 d)		(1-H)
		11-OH (3.687)		(11-H)
		12-OH 7.449 s (5.361 d)		12-H
		14-OH (5.157 s)		
		15-OH (5.373 d)		(15-H)

[a] The spectra were taken on a Varian XL-400 (^{1}H, 400 MHz, ^{13}C, 100.6 MHz) in pyridine-d_5, ppm. [b] These data were obtained by ^{1}H-^{13}C COSY experiments. [c] These data were obtained by ^{1}H-^{1}H COSY experiments. The data in parentheses were additionally obtained in DMSO-d_6. Coupling constants (Hz) were as follows: $J_{5,6\alpha} = 2.7$, $J_{5,6\beta} = 12.8$, $J_{6\alpha,\beta} = 14.5$, $J_{6\alpha,7} = J_{6\beta,7} = 2.7$, $J_{9,11} = 4.5$, $J_{20A,B} = 7.0$, ($J_{1,1\text{-OH}} = 3.3$, $J_{11,11\text{-OH}} = 5.5$, $J_{12,12\text{-OH}} = 4.7$, $J_{15,15\text{-OH}} = 5.6$). [d] The data in parentheses were obtained in DMSO-d_6.

quired to be 8β(ax)-C(20) and 13β(ax)-O(21) [13α(eq)-CH_2OH] [9] with respect to C-ring regardless of its conformation. Irradiation of 12-OH (DMSO-d_6) indicated an nOe at 9-H (d, J = 4.5 Hz) which interacted with 15-H each other, leading to 9α(ax)-H, 12α(ax)-OH [12β(eq)-H] and 14α(ax)-C(15) [14β(eq)-OH] with respect to C-ring with a chair form (*vide infra*). As a result, the B/C *trans* and C/D *cis* ring junctures were established as shown in structural fragment **A** (Scheme 1). An nOe observed between 9α-H and 11-H and a coupling constant, $J_{9,11}$ = 4.5 Hz, suggested to be 11α(eq)-H and 11β(ax)-OH. No coupling observed between 11α-H and 12β-H (dihedral angle, *ca.* 90°) and an nOe observed between 12β-H and 20-H_B showed the slight deformation of C-ring to relieve the strain caused by two α(axial)-and three β(axial)-substituents. An nOe observed between 7-H and 20-H_A and coupling constants, $J_{6\alpha,7} = J_{6\beta,7}$ = 2.3 Hz, showed to be 7β(eq)-H with respect to B-ring with a chair form and the B/D *cis* ring juncture (fragment **A**). It is known that δ-lactones adopt a half-chair or a half-boat form with a planar C-C(=O)-O-C moiety [10]. A dreiding model showed that a half-boat form of D-ring enlarges the distance between C(20) and O(21), and the formation of an ether bridge between C(13) and C(20) sterically becomes impossible. Thus, D-ring adopts a half-chair form which is supported by the ir spectrum (chloroform) showing a δ-lactone carbonyl at 1727 cm^{-1} [10]. NOes observed among 9α-H, 12α-OH and 15-H (*vide supra*) led to 15α(ax)-H and 15β(eq)-OH with respect to D-ring (fragment **A**). A further evidence supporting the B/D and C/D *cis* ring junctures was obtained by the observations of nOes at 7β-H and 20-H_A on irradiation of 14β-OH (DMSO-d_6).

Table III

NMR Data for Bruceine E (**4**) [a]

No	Carbon δ c	Correlated proton [b] One-bond [d]	Long-range	^1H-^1H [c]
1	82.768 d	1-H 3.973 d (3.370 m)	2-H, 3-H 18-H_3	2-H (1-OH)
2	73.606 d	2-H 4.457 m (3.799 m)	1-H	1-H, 3-H 5-H, 19-H_3 (2-OH)
3	126.468 d	3-H 5.618 br s	19-H_3	2-H, 5-H 19-H_3
4	134.970 s		5-H, 19-H_3	
5	43.345 d	5-H 2.599 br d (2.240 d)	3-H, 7-H 11-H, 18-H_3 19-H_3	2-H, 3-H 6β-H, 18-H_3 19-H_3
6	28.395 t	6α-H 2.080 dt (1.928 dt) 6β-H 1.507 ddd	5-H	6β-H, 7-H (5-H) 5-H, 6α-H 7-H
7	80.668 d	7-H 5.373 t (4.868 t)	11-H	6α-H, 6β-H 9-H
8	50.732 s		6α-H, 7-H 20-H_A	
9	46.654 d	9-H 2.568 d (1.898 d)	5-H, 11-H 12-H, 18-H_3 20-H_A	7-H, 11-H 20-H_A
10	45.171 s		6α-H, 6β-H 18-H_3	
11	76.300 d	11-H 5.362 d (4.369 t)	9-H, 12-H	9-H (11-OH)
12	81.654 d	12-H 4.510 s (3.523 d)	11-H, 22-H_3	12-OH
13	84.647 s		11-H, 12-H 22-H_3	
14	82.768 s		12-H, 15-H 22-H_B, 22-H_3	
15	70.662 d	15-H 5.863 s (5.343 d)		(15-OH)
16	175.421 s		6β-H, 15-H	
18	12.727 q	18-H_3 1.438 s (1.068 s)	1-H	5-H
19	21.213 q	19-H_3 1.438 s (1.558 s)	3-H	2-H,3-H
20	70.599 t	20-H_A 4.158 20-H_B 5.002 d	9-H	9-H, 20-H_B 20-H_A
22	19.656 q	22-H_3 1.949 s (1.227 s)	12-H	
		1-OH (5.452 d)		(1-H)
		2-OH (4.658 d)		(2-H)
		11-OH, (3.984 d)		(11-H)
		12-OH 7.201 d (5.275 d)		12-H
		14-OH (5.081 s)		
		15-OH (4.860 d)		(15-H)

[a] The spectra were taken on a Varian XL-400 (^1H, 400 MHz, ^{13}C, 100.6 MHz) in pyridine-d_5, ppm. [b] These data were obtained by ^1H-^{13}C COSY experiments. [c] These data were obtained by ^1H-^1H COSY experiments. The data in parentheses were additionally obtained in DMSO-d_6. Coupling constants (Hz) were as follows: $J_{1,2}$ = 7.3, $J_{5,6\alpha}$ = 2.5, $J_{5,6\beta}$ = 13.0, $J_{6\alpha,\beta}$ = 14.7, $J_{6\alpha,7} = J_{6\beta,7}$ = 2.5, $J_{9,11}$ = 5.0, $J_{20A,B}$ = 6.8, ($J_{1,1\text{-OH}}$ = 4.0, $J_{2,2\text{-OH}}$ = 6.2, $J_{11,11\text{-OH}}$ = 5.3, $J_{12,12\text{-OH}}$ = 5.0, $J_{15,15\text{-OH}}$ = 6.0). [d] The data in parentheses were obtained in DMSO-d_6.

Irradiation of 1-H afforded nOes at 5-H (br d, J = 12.7 Hz) and 9α-H. An nOe was observed between 18-H_3 and 20-H_B. These observations suggested that the A/B ring juncture exists in the *trans* configuration with 1α(ax)-H [1β(eq)-OH], 5α(ax)-H and 10β(ax)-Me and that A-ring adopts a half-chair or an envelope or a half-boat form and B-ring a chair form (*vide supra*). At this stage, 6β(ax)-H was identified by an nOe observed on irradiation of 18-H_3 and a coupling constant, $J_{5,6\beta}$ = 12.7 Hz. Irradiation of 11α-H gave nOes at 1α-H and 9α-H, providing a further support to be 11β(ax)-OH.

Dreiding model **1b** [11] (Scheme 1) based on the above-obtained data clearly accounts for the additional data presented in Tables I and IV.

Bruceine D (**2**).

The assignments of protons and carbons were made in the same manner as employed above (Table II). The relative stereochemistry of **2** was establisehd to be the same (Dreiding model **2b** in Scheme 1) as that of **1** on the basis of the similarity of their nOe and ^1H-^1H decoupling data (Tables II and V).

Bruceine E (**4**).

The assignments of protons and carbons were made in the same manner as employed above (Table III). Because of the close absorptions due to several protons (2-H and 12-H; 5-H and 9-H; 7-H and 11-H; 18-H_3 and 19-H_3), the discreet cautions were paid to analyze nOes observed among these protons. When simultaneously irradiated or observed at two protons, the interacting protons were decided by taking into account their internuclear distances obtained from Dreiding model [12]. The relationship

Table IV

NOE Data for Bruceine H (**1**)

Irradiated Proton	Observed Proton(NOE %)
1-H	5-H (7.4%), 9-H (5.7%), 11-H (2.5%)
5-H	1-H (5.4%), 6α-H (3.0%), 9-H (2.9%)
7-H	6α-H (1.5%), 6β-H (2.9%), 20-H_A (4.3%)
9-H	1-H (7.9%), 5-H (7.4%), 11-H (5.8%), 15-H (15.7%)
11-H	1-H (5.0%), 9-H (4.3%), 12-H (5.3%), 18-H_3 (1.1%)
12-H	11-H (1.9%), 22-H_B (3.9%)
15-H	9-H (12.9%), 22-H_A (1.9%)
18-H_3	6β-H (7.9%), 11-H (1.3%), 20-H_B (6.6%)
20-H_A	7-H (6.3%), 20-H_B (11.8%)
20-H_B	6β-H (3.3%), 12-H (1.9%), 18-H_3 (2.7%), 20-H_A (18.6%)
22-H_A	15-H (7.1%), 22-H_B (9.2%)
22-H_B	12-H (1.3%), 22-H_A (5.2%)
12-OH	9-H (4.1%) [a], 12-H (2.7%), 22-H_A (1.9%)
14-OH	7-H (1.6%) [a], 20-H_A (2.0%) [a]

[a] These data were obtained in DMSO-d_6.

Table V

NOE Data for Bruceine D (**2**)

Irradiated Proton	Observed Proton(NOE %)
1-H	5-H (6.7%), 9-H (4.8%), 11-H (1.5%)
5-H	1-H (4.6%), 6α-H (2.3%), 9-H (3.2%)
7-H	6α-H (3.1%), 20-H_A (3.1%), 14-OH (1.2%) [a]
9-H	1-H (5.1%), 5-H (4.0%), 11-H (4.6%), 15-H (8.6%)
11-H	1-H (2.3%), 9-H (3.2%), 12-H (5.1%)
12-H	11-H (2.3%), 22-H_3 (0.6%)
15-H	9-H (9.5%), 22-OH (1.6%) [a]
18-H_3	6β-H (3.8%), 11-H (1.3%), 20-H_B (6.2%)
20-H_A	7-H (4.6%), 20-H_B (10.8%), 14-OH (1.2%) [a]
20-H_B	12-H (1.7%), 18-H_3 (3.3%), 20-H_A (10.0%)
22-H_3	12-H (5.1%)
12-OH	9-H (4.4%) [a], 12-H (5.1%)
14-OH	7-H (1.3%) [a], 20-H_A (1.0%) [a]

[a] These data were obtained in DMSO-d_6.

Table VI

NOE Data for Bruceine E (**4**)

Irradiated Proton	Observed Proton(NOE %)
1-H	5-H (2.7%) [a], 9-H (3.7%) [a], 11-H (6.3%)
2-H	3-H (8.1%), 18-H_3 (1.7%)
7-H	6α-H (3.2%), 6β-H (3.6%), 20-H_A (3.1%), 20-H_B (2.5%)
9-H	11-H (3.8%), 15-H (10.0%)
11-H	1-H (2.5%), 9-H (5.8%), 12-H (5.5%), 18-H_3 (1.5%)
12-H	11-H (5.0%), 22-H_3 (2.0%)
15-H	9-H (5.1%)
18-H_3	2-H (5.6%), 6β-H (1.9%) [a], 20-H_B (5.4%)
19-H_3	3-H (3.8%), 5-H (1.9%), 6α-H (3.2%)
20-H_A	7-H (5.0%), 20-H_B (10.0%)
20-H_B	6β-H (2.4%) [a], 12-H (2.7%), 18-H_3 (1.0%), 20-H_A (10.0%)
22-H_3	12-H (6.8%), 15-H (1.9%), 12-OH (3.1%), 14-OH (7.1%) [a]
12-OH	9-H (5.0%), 11-H (3.1%), 12-H (8.2%), 15-H (4.6%)
14-OH	20-H_A (3.7%) [a], 22-H_3 (1.8%) [a]

[a] These data were obtained in DMSO-d_6.

among 1-H, 5-H and 9-H was examined in DMSO-d_6 solution, in which the signals of the latter two were well separated. It became apparent that the nOe and ^1H-^1H decoupling data for **4** are similar to those for **1** (Table III and VI). Thus, the relative stereochemistry, except the 2-OH configuration, was easily assigned to be the same as that of **1** in the same manner as employed above, being in accord with that the oxidation of **4** furnished **2** [2]. Finally, an nOe observed between 2-H ($J_{1,2}$ = 7.3 Hz), and 18-H_3 suggested to be 2β(ax)-H [2α(eq)-OH] and a half-chair form (Dreiding model **4b** in Scheme 1) for A-ring.

CD Spectroscopy.

Bruceine H (**1**) and D (**2**).

It is well known that the cd spectra of α,β-unsaturated ketones show the characteristic $n\rightarrow\pi^*$ Cotton effects (CEs), the signs of which are due to the ring conformations containing a C=C-C=O moiety [13]. A number of Δ^1-3-oxo-5α-steroids with the A/B *trans* ring juncture, in which A-ring adopted an envelope or a half-boat form, provided negative $n\rightarrow\pi^*$ CEs [13,14]. Snatzke [13,15] pictorially correlated the sign of $n\rightarrow\pi^*$ CEs with the conformations of A-ring as shown in drawings (Figure 5). The bond C(4)-C(5) in the right lower back octant of an envelope form (left) and C(1)-C(2) in the left upper one of a half-boat form (right) give a negative contribution to $n\rightarrow\pi^*$ CEs. In spite of the similarity of A-ring, ailanthone (**5**) (5*S*, 10*S*) [16] and 13,22-dehydroglaucarubinone (**6**) (5*S*, 10*S*) [17] indicated CEs $[\theta]_{310}$ +977 and $[\theta]_{320}$ +3498, respectively. X-Ray analysis of the analogous quassinoids [16-18] showed that A-ring adopted a half-chair form. If it is assumed that A-ring in **5** and **6** also adopts a half-chair form in solution, the observed positive $n\rightarrow\pi^*$ CEs are explainable as shown in a drawing (Figure 6) which is a mirror image of a drawing (right) in Figure 5. Since it seems probably that the conformations of A-ring in **1** and **2** are the same as those in **5** and **6**, positive $n\rightarrow\pi^*$ CEs are expected for **1** and **2**. Actually, the cd spectra of **1** and **2** furnished CEs $[\theta]_{314}$ +6816 and $[\theta]_{311.5}$ +3972, respectively, suggesting to be the same configuration (5*S*, 10*S*) as that of **5** and **6** (Figure 7).

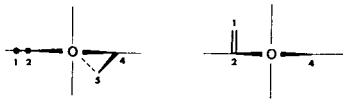


Figure 5. Sign of $n\rightarrow\pi^*$ CE and conformations of A-ring in Δ^1-3-oxo-5α-steroids (A / B trans).

In order to obtain further support, the cd spectrum of 3,4-dihydrobruceine D (**7**), which was derived from **2** by the hydrogenation over palladium-carbon, was examined. NOes observed between 1α-H and 3α-H and between 18-H_3 and 19-H_3, and coupling constants, $J_{3\alpha,4}$ = 7.5, $J_{3\beta,4}$ = 1.5 and $J_{4,5\alpha}$ = 5.0 Hz, suggested that A-ring in **7** adopts a slightly flattened chair form with 4β(ax)-Me owing to 1,3-diaxial interaction between 4β-Me and 10β-Me. A CE $[\theta]_{282.5}$ +1712 observed for **7** was confirmed to be the 5*S*, 10*S*-configuration by the application of the well-known Octant rule (Figure 8).

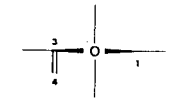


Figure 6. Sign of $n\rightarrow\pi^*$ CE and conformation of A-ring in bruceines H (1) and D (2) and related quassinoids 5 and 6.

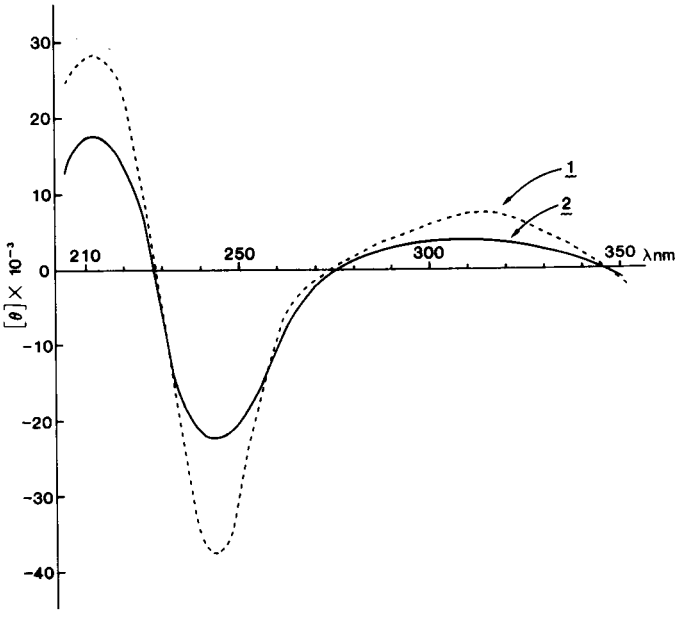


Figure 7. Cd curves of bruceines H (1) and D (2).

Thus, the configurations at the remaining asymmetric centers in **1** and **2** are assigned to be 1*S*, 7*R*, 8*R*, 9*R*, 11*R*, 12*S*, 13*R*, 14*R*, 15*R* on the basis of the relative stereochemistry.

Bruceine E (**4**).

Wolf [19] reported that the cd spectra of δ-lactones showed the characteristic $n\rightarrow\pi_3^*$ CEs, the signs of which were due to the ring conformations adopting a half-chair or a half-boat form with a planar C-C(=O)-O-C moiety. A CE $[\theta]_{227.5}$ −2622 was observed for **4** with D-ring adopting a half-chair form (*vide supra*) (Figure 8). When D-ring depicted according to Wolf's rule contains C(7)-C(8) and C(14)-C(15) in the upper and lower sides, respectively, of

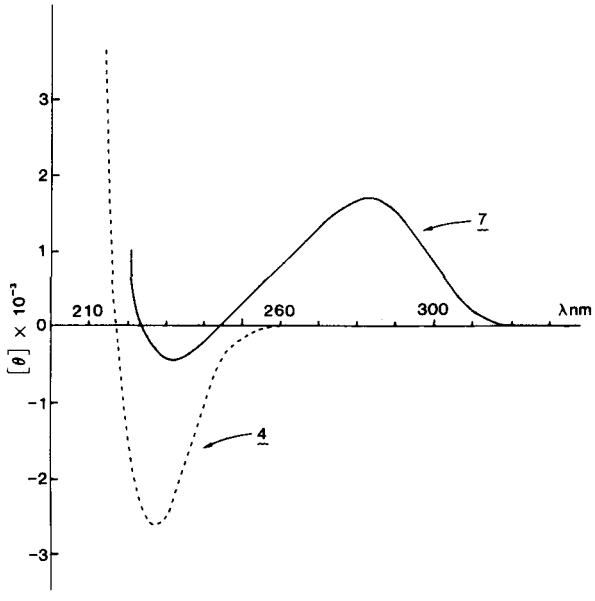


Figure 8. Cd curves of bruceine E (4) and 3,4-dihydrobruceine D (7).

the C-C(=O)-O-C plane, a negative $\pi \rightarrow \pi_3^*$ CE is observed for **4** (Figure 9). This geometry is in accord with the 7*R*, 8*R*, 14*R*, 15*R*-configuration. This finding leads to the same absolute stereochemistry with the 2*S*-configuration for **4** as that of **1** and **2** on the basis of the relative stereochemistry.

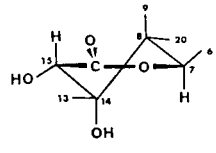


Figure 9. Sign of $\pi \rightarrow \pi_3^*$ CE and conformation of D-ring in bruceine E (4).

Now, the absolute stereostructures **1c**, **2c** and **4c**, which were conventionally used for **1**, **2** and **4** for a long time, have been confirmed to be correct by means of nmr and cd spectroscopic analyses.

In order to ascertain the above conclusion, X-ray analysis of **1** is being prepared in our laboratories.

EXPERIMENTAL

Melting points were determined on a micro hot-stage apparatus and are uncorrected. Specific rotations were taken on a JASCO DPI-181 polarimeter. Spectra were recorded on the following specrometers: uv, Hitachi EPS-2U; cd, JASCO J-20; ir, Hitachi 260-30; ^{1}H nmr, Varian VXR-300 and XL-400 at 300 and 400 MHz; ^{13}C nmr, Varian XL-400 at 100.6 MHz; ms, JEOL JMS DX-300; elemental analysis, Perkin-Elmer 240B. All nmr spectra were taken at a probe temperature, 20°, using a 5 mm tube.

The ^{1}H-^{13}C COSY experiments for **1**, **2** and **4** were run under the following conditions. The conditions for **2** and **4** are given in parentheses, respectively. The 90° ^{1}H and ^{13}C pulses were calibrated at 31.0 and 9.7 μsec, respectively. One-bond correlation: sweep width, 3086 (2030, 2547) Hz in the F_1 domain (^{1}H) (memory size, 512 (256, 256)) and 19418 (14451, 16949) Hz in the F_2 (^{13}C) (memory size, 2K); quadrature collection; relaxation delay, 1 sec; $^1J_{CH}$ (average), 140 Hz; number of scans, 64 (48, 64) (transients, 32 (32, 16)); size of final data points, 2K; acquisition time, 0.053 (0.071, 0.060) sec. Long-range correlation: sweep width, 3086 (3150, 2547) Hz in the F_1 domain (^{1}H) (memory size, 512 (512, 256)) and 19417 (19960, 16949) Hz in the F_2 domain (^{13}C) (memory size, 2K); quadrature colllection; relaxation delay, 1 sec; $^{LR}J_{CH}$ (average), 7.0 Hz; number of scans, 80 (64, 64) (transients, 2560 (2816, 704)); size of final data points, 2K; acquisition time, 0.053 (0.051, 0.060) sec.

The nOe spectra (400 MHz) were recorded in the degassed solutions by means of nOe difference spectroscopy. The pre-irradiation time of each resonance was 2 seconds. The interpulse delay was zero sec. The irradiation data sets were interleaved to cancel drift and changing magnet homogenity. These spectra were transformed by the difference between two free induction decays.

Bruceine H (**1**).

Compound **1** was obtained as colorless needles, mp 285-287° (lit [5] mp 285-287°); Rf 0.34 (Silica gel, chloroform-methanol = 5.1, v/v); specific rotation: $[\alpha]_D^{23}$ +48.8° (c = 0.53, methanol); uv (methanol): λ (ε) 294 (133.1), 236 (13276.5); cd: $[\theta]^{25}$ (nm) +6818 (314.0) (positive maximum), 0 (273.5), −37772 (243.2) (negative maximum), 0 (228.0), +28400 (212.0) (positive maximum) (c = 1.5×10^{-5}, methanol); ir (chloroform): ν cm^{-1} 1727 (lactone), 1658 (-C=C-C=O); ms: m/z M$^+$, 426.1492 (M, 426.1525).

Anal. Calcd. for $C_{20}H_{26}O_{10}\cdot\frac{1}{2}H_2O$: C, 55.17; H, 6.25. Found: C, 55.27; H, 6.29.

Bruceine D (**2**).

Compound **2** was obtained as colorless needles, mp 294-295° (lit [2] mp 285-290°); Rf 0.52 (Silica gel, chloroform-methanol = 5:1, v/v); specific rotation: $[\alpha]_D^{23}$ +25.9° (c = 0.34, methanol); uv (methanol): λ (ε) 299 (887.2), 238 (14510.3); cd: $[\theta]^{25}$ (nm) +3972 (311.5) (positive maximum), 0 (275.0), −22596 (243.0) (negative maximum), 0 (228.0), +18223 (212.0) (positive maximum) (c = 4.5×10^{-5}, methanol); ir (chloroform): ν cm^{-1} 1727 (lactone), 1664 (-C=C-C=O); ms: m/z M$^+$, 410.1589 (M, 410.1576).

Anal. Calcd. for $C_{20}H_{26}O_9$: C, 58.53; H, 6.39. Found: C, 58.73; H, 6.51.

Bruceine E (**4**).

Compound **4** was obtained as colorless needles, mp 257-260° (lit [2] mp 260-264°), R*f* 0.32 (Silica gel, chloroform-methanol = 5:1, v/v); specific rotation: $[\alpha]_D^{23}$ +55.5° (c = 0.53, methanol); uv (methanol): λ (ε) 246 (72.3); cd: $[\theta]^{25}$ (nm) 0 (260), −2622 (227.5) (negative maximum), 0 (217) (c = 5.5×10^{-4}, methanol); ir (chloroform): ν cm^{-1} 1710 (lactone), 1590 (>C=C<); ms: m/z M$^+$, 412.1749 (M, 412.1733).

Anal. Calcd. for $C_{20}H_{28}O_9\cdot H_2O$: C, 55.81; H, 7.02. Found: C, 56.08; H, 7.13.

3,4-Dihydrobruceine D (**7**).

A solution of **2** (3 mg) in anhydrous ethanol (5 ml) was hydroge-

nated over palladium-carbon (3 mg) at ambient temperature for 1 hour. Work-up of the reaction mixture, followed by preparative tlc (silica gel, chloroform-methanol = 5.1, v/v) gave **7** (2.5 mg, 83%) as colorless needles, mp 205-207° (ethyl acetate); Rf 0.40 (silica gel, chloroform-methanol = 5:1, v/v); specific rotation: $[\alpha]_D^{25}$ +27.6° (c = 0.12, methanol); uv (methanol): λ (ε) 280 (1900), 238 (4600); cd: $[\theta]^{25}$ (nm) 0 (319.5), +1712 (282.5) (positive maximum), 0 (244.5), −412 (233.0) (negative maximum), 0 (222.0) (c = 7.5 × 10^{-4}, methanol); ir (chloroform): ν cm^{-1} 1715 (lactone), 1702 (>C=O); ^1H nmr (300 MHz, pyridine-d_5): δ 0.708 (3H, d, J = 7.5 Hz, 19-H_3), 1.360 (3H, s, 18-H_3), 1.689 (1H, dt, J = 14.8, 2.7 Hz, 6α-H), 1.765 (1H, ddd, J = 14.8, 13.0, 2.7 Hz, 6β-H), 1.940-2.060 (1H, m, 4-H), 1.964 (3H, s, 22-H_3), 2.218 (1H, dd, J = 13.0, 1.5 Hz, 3β-H), 2.439 (1H, ddm, J = 13.0, 5.0 Hz, 5-H), 2.573 (1-H, d, J = 5.5 Hz, 9-H), 2.642 (1H, dd, J = 13.0, 7.5 Hz, 3α-H), 4.125 (1H, d, J = 7.0 Hz, 20-H_A), 4.225 (1H, d, J = 2.7 Hz, 1-H) [20], 4.483 (1H, d, J = 5.5 Hz, 12-H) [20], 4.641 (1H, d, J = 7.0 Hz, 20-H_3), 5.038 (1H, t, J = 5.5 Hz, 11-H) [21], 5.362 (1H, t, J = 2.7 Hz, 7-H), 5.936 (1H, br s, 15-H), the hydroxyl signals were obscure; nOe: 3α-H → 1-H (3.3%), 5-H (0.5%); 18-H_3 → 6β-H (6.6%), 19-H_3 (1.9%); 19-H_3 → 3β-H (3.2%), 6β-H (3.4%), 18-H_3 (2.2%); ms: m/z M^+, 412.1704 (M, 412.1733).

Anal. Calcd. for $C_{20}H_{28}O_9 \cdot \frac{1}{4}H_2O$: C, 57.62; H, 6.89. Found: C, 57.80; H, 7.12.

Acknowledgement.

The authors thank Dr. H. Komura (Suntory Institute for Bioorganic Research) for the measurements of cd spectra.

REFERENCES AND NOTES

[1] J. Polonsky, Z. Baskevitch, A. Gaudemer, and B. C. Das, *Experientia*, **23**, 424 (1967); G. R. Duncan and D. B. Henderson, *ibid.*, **24**, 768 (1968); K. Y. Sim, J. J. Sims, and T. A. Geissman, *J. Org. Chem.*, **33**, 429 (1968); J. Polonsky, J. Varenne, T. Prange, and C. Pascard, *Tetrahedron Letters*, **21**, 1853 (1980).

[2] J. Polonsky, Z. Baskevitch, B. C. Das, and J. Müller, *C. R. Seances Acad. Sci., Ser. C*, **267**, 1346 (1968).

[3] K.-H. Lee, Y. Imakura, Y. Simida, R.-Y. Wu, and I. H. Hall, *J. Org. Chem.*, **44**, 2180 (1979).

[4] W. Stöcklin and T. A. Geissman, *Tetrahedron Letters*, 6007 (1968).

[5] S. Yoshimura, T. Sakaki, M. Ishibashi, T. Tsuyuki, T. Takahashi, K. Matsushita, and T. Handa, *Chem. Pharm. Bull.*, **32**, 4698 (1984).

[6] J. Polonsky, Z. Baskevitch, and J. Müller, *C. R. Seances Acad. Sci., Ser. C*, **268**, 1392 (1969).

[7] X. Li, Y. Lin, S. Wang, and Z. Zhu, *J. Shenyang Coll. Pharm.*, **3**, 192 (1986). Because of insufficiency in amounts, bruceine F could not be used for the reference sample.

[8] J. Polonsky, *Fortschr. Chem. Org. Naturst.*, **30**, 101 (1973).

[9] α and β notations are tentatively used until the absolute stereochemistry is made clear.

[10] K. K. Cheung, K. H. Overton, and G. A. Sim, *J. Chem. Soc., Chem. Commun.*, 634 (1965); C. Pascard and J. Polonsky, *J. Chem. Res. (S)*, 324 (1977).

[11] The A-ring was shown in a half-chair form for convenience.

[12] R. A. Bell and J. K. Saunders, *Can. J. Chem.*, **48**, 1114 (1970); G. E. Bachers and T. Schaefer, *Chem. Rev.*, **71**, 617 (1971); N. C. Gonnella and J. Chen, *Magn. Reson. Chem.*, **26**, 185 (1988).

[13] G. Snatzke, "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry", G. Snatzke, ed, Heyden and Son Ltd., London, 1967, p 208, and references cited therein.

[14] P. Witz, H. Herrman, J.-M. Lehn, and G. Ourisson, *Bull. Soc. Chim. France*, 1101 (1963); G. Snatzke, *Tetrahedron*, **21**, 421 (1965).

[15] G. Snatzke, *Tetrahedron*, **21**, 413 (1965).

[16] H. Naora, M. Ishibashi, T. Furuno, T. Tsuyuki, T. Murae, H. Hirota, T. Takahashi, A. Itai, and Y. Iitaka, *Bull. Chem. Soc. Japan*, **56**, 3694 (1983).

[17] J. Polonsky, Z. Varon, H. Jacquemin, and G. R. Pettit, *Experientia*, **34**, 1122 (1978); G. Kartha and D. J. Haas, *J. Am. Chem. Soc.*, **86**, 3630 (1964).

[18] M. C. Wani, H. L. Taylor, J. B. Thompson, and N. E. Wall, *Tetrahedron*, **35**, 17 (1979); J. Polonsky, M. V. Tri, Z. Varon, T. Prange, and C. Pascard, *ibid.*, **36**, 2983 (1980).

[19] H. Wolf, *Tetrahedron Letters*, 1075 (1965); *ibid.*, 5151 (1966). It is known that the application of the sector rule to δ-lactones occasionally leads to the confused results [J. P. Jennings, W. Klyne, and P. M. Scopes, *J. Chem. Soc.*, 7211 (1965); W. Klyne, and P. M. Scopes, "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry", G. Snatzke, ed, Heyden and Son Ltd., London, 1967, p 193 and references cited therein].

[20] On addition of deuterium oxide, this signal changed to a singlet.

[21] On addition of deuterium oxide, this signal changed to a doublet.