Relative Population of S-Form and F-Form Conformers of Bryonolic Acid and Its Derivatives in Equilibrium in $CDCl_3$ Solutions

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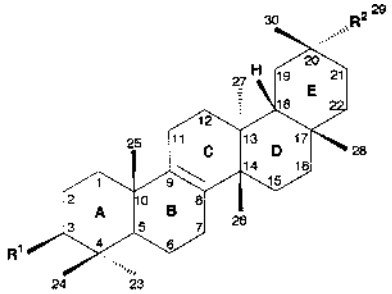
Relative populations of S-form (D–E rings: boat–boat form) and F-form (D–E rings: chair–chair form) conformers, in equilibrium in $CDCl_3$ solutions, of 20 derivatives (2—21) of bryonolic acid (D:C-friedoolean-8-en-3β-ol-29-oic acid) (1) were calculated from NMR spectral data (*J*-values and chemical shifts), with the aid of molecular mechanic calculation using a MM2/CONFLEX program system. The principal deciding factor of the population ratio was found to be whether the functionality at C-29 is trigonal or tetrahedral; the S-form : F-form was 0 : 100—32 : 68 for the "trigonal" type and 48 : 52—100 : 0 for the "tetrahedral." The reliability of the results is discussed.

Key words bryonolic acid; conformation; MM2 calculation; CONFLEX

Bryonolic acid (D:C-friedoolean-8-en-3β-ol-29-oic acid) (**1**)[1] (see Fig. 1) is a D:C-friedooleanane class of triterpene which has been isolated from the roots, cultured cells or cultured hairy roots of various cucurbitacean plants.[2] Previously, Kamisako *et al.* suggested that two types of conformations (Fig. 2), *i.e.*, the S-form (D–E rings: boat–boat form) and the F-form (D–E rings: chair–chair form) are possible for bryonolic acid derivatives, that molecules having these conformations co-exist in equilibrium in $CDCl_3$ solutions, and that their relative populations are greatly affected by the functionality at C-29.[3]

Tabata and co-workers found that **1** had anti-allergic activities.[4] They also showed that the anti-allergic activities were significantly affected by the C-29 functionality; converting the functional group at C-29 from –COOH to $-CH_2OCO-(CH_2)_2COONa$ raised the activities as much as by tenfold.[5] More recently, a variety of biological or pharmacological activities, such as anti-inflammatory,[6] sebum secretion promoting,[7] antitumor[8] and skin cell growth promoting action,[9] have been reported by other workers. This led us to conduct more detailed studies on the conformational feature of **1** and its derivatives. The physicochemical properties described above should be taken into account when relationships between biological activity and structure are discussed.

This paper presents studies on the relationship between the C-29 functionality and relative populations of the S-form and F-form conformers in equilibrium in solutions (S : F populations). Twenty model compounds (**2**—**21**) (see Fig. 1) were examined and their S : F populations were calculated from the NMR spectral data, *i.e.*, *J*-values and chemical shifts, with the aid of molecular mechanics calculation using an MM2 program.[10] CONFLEX,[11] a program utilized together with the MM2, enabled us to efficiently find all the practical conformers, such as rotamers around the C(20)—C(29) bonds of both the S-form and F-form conformers, and to check the probability of other conformational types corresponding to those previously discussed by Kikuchi and co-workers[12] The MM2 calculation also offered us theoretical



	R^1	R^2		R^1	R^2
1	OH	COOH	12	$OCOCH_3$	$CH_2O\overset{31}{C}O\overset{32}{C}H_3$
2	$OCOCH_3$	CHO	13	$OCOCH_3$	CH_2OH
3	H	COOH	14	OH	$CH_2\overset{31}{C}H_3$
4	OH	$CO\overset{31}{O}CH_3$	15	$OCOCH_3$	$CH<^{O}_{O}>^{31}_{32}$
5	OH	$CH=NNH_2$	16 [b]	OH	$CH(OH)\overset{31}{C}H_3$
6	$OCOCH_3$	$CO\overset{31}{C}H_3$	17 [b]	OH	$CH(OH)\overset{31}{C}H_3$
7	OH	$C(=NNH_2)\overset{31}{C}H_3$	18	$OCOCH_3$	$CH(\overset{31}{C}H_3)_2$
8	OH	$CH=\overset{31}{C}H_2$	19	$OCOCH_3$	$\overset{31}{C}(CH_3)<^{O}_{O}>^{32}_{33}$
9	$OCOCH_3$	$CH=\overset{31}{C}H_2$	20	OH	$C(OH)(\overset{31}{C}H_3)_2$
10	$OCOCH_3$	$C(=\overset{31}{C}H_2)\overset{32}{C}H_3$	21	$OCOCH_3$	$C(O\overset{31}{C}O\overset{32}{C}H_3)(\overset{33}{C}H_3)_2$
11	OH	CH_3			

Fig. 1. Structures of Bryonolic Acid (**1**) and Its Derivatives (**2**—**21**)[a]

a) Extra numbers 31—34 are given to the carbons and protons of the substituents at C-29. *b*) Compounds **16** and **17** are epimeric to each other with respect to the asymmetric center at C-29.

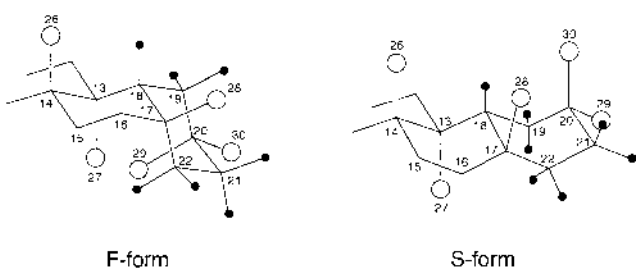


Fig. 2. Conformations of the D–E Ring Systems of D:A—D:C-Friedooleananes Drawn from the Dreiding Model in a Regular Conformation

Open circles, methyl group; closed circles, hydrogen atoms.

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Table 1. ^{13}C Chemical Shifts of Bryonolic Acid Derivatives[a)]

Carbon	2	3	4	5	6	7	8	9	10	11
1	34.73	36.88	35.11	35.23	34.81	35.39	35.13	34.79	34.82	35.10
2	24.23	19.29	27.96	28.09	24.24	28.05	28.00	24.27	24.26	27.99
3	80.89	41.73	78.99	79.36	80.93	79.30	79.07	81.00	81.01	79.07
4	37.79	33.31	38.85	38.91	37.78	38.88	38.85	37.78	37.77	38.85
5	50.58	51.46	50.56	50.53	50.66	50.57	50.70	50.77	50.85	50.78
6	19.08	19.49	19.25	19.07	19.11	19.15	19.27	19.14	19.15	19.30
7	27.41	27.78	27.60	28.29	27.42	28.05	27.61	27.45	27.38	27.53
8	134.10	133.31	133.95	134.21	134.22	134.16	134.51	134.60	134.98	135.16
9	133.87	134.80	134.08	133.70	133.83	133.92	133.89	133.71	133.50	133.59
10	37.46	37.87	37.59	37.66	37.47	37.62	37.62	37.48	37.50	37.67
11	20.63	20.80	20.70	20.75	20.69	20.82	20.77	20.77	20.82	20.84
12	30.85	30.19	30.40	30.44	30.59	30.75	30.74	30.17	30.57	30.75
13	37.42	37.08	37.23	37.19	37.33	37.09	37.36	37.34	37.50	37.36
14	41.84	42.10	41.88	42.15	41.72	41.84	41.52	41.52	41.19	41.02
15	25.00	25.62	25.10	25.73	25.29	25.88	25.59	25.59	26.15	26.35
16	36.99	37.03	37.08	37.38	36.89	37.37	37.11	37.08	36.77	36.73
17	30.91	30.93	30.95	30.78	31.06	31.10	31.02	31.00	31.28	30.84
18	44.32	44.64	44.84	45.02	44.66	45.37	44.96	44.95	44.58	44.16
19	29.25	30.51	30.82	33.01	30.20	30.64	34.37	34.36	30.97	34.49
20	44.45	40.46	40.51	36.09	46.44	39.74	34.77	34.73	37.11	28.35
21	26.37	29.61	29.98	29.13	29.11	30.40	30.14	30.72	31.22	34.21
22	34.10	34.32	34.46	34.64	34.79	35.29	35.24	35.24	36.58	36.90
23	28.00	33.02	28.05	27.76	28.01	27.83	28.08	28.02	28.03	28.09
24	16.68	21.89	15.57	15.74	16.69	15.68	15.58	16.68	16.70	15.62
25	19.95	19.88	19.91	19.77	19.96	19.81	19.90	19.95	19.93	19.85
26	22.33	21.81	22.08	21.70	22.53	22.19	23.05	23.07	24.21	24.77
27	19.04	18.18	17.17	21.43	18.50	19.58	20.58	20.48	18.76	18.97
28	31.14	31.31	31.24	31.84	31.20	31.88	31.63	31.63	31.50	31.52
29	205.27	185.46	179.21	174.25	213.95	169.13	151.35	151.25	153.56	33.06
30	27.62	32.73	32.87	31.37	31.50	32.49	34.30	34.25	32.53	34.45
31			51.49		25.36	13.84	107.74	107.82	108.53	
32									19.96	
33										
34										
3-O<u>C</u>OCH$_3$	170.88				170.91			170.95	170.94	
3-OCO<u>C</u>H$_3$	21.28				21.28			21.29	21.29	

grounds for consideration of the reliability of the concluded S : F populations.

Methods and Results

We synthesized **3**, **4**, **12** and **13** described in previous papers,[3,13)] and also 16 derivatives, **2**, **5**—**11** and **14**—**21**. C-29 functional groups of these model compounds were designed to allow us to reveal the effect on the S : F population of C-29 functionality, *i.e.*, whether the functional groups are trigonal or tetrahedral and the identity of the substituents linking to the C-29 carbon atoms.

Assignments of carbon chemical shifts of **2**—**21** were done by comparing the shift data with those of the related compounds[3,13)] and recording distortion enhancement by polarization transfer (DEPT) and ^1H-detected heteronuclear multiple bond correlation (HMBC) spectra. ^1H-noise decoupled ^{13}C spectra of several compounds, which were derived from biosynthetically ^{13}C-enriched species of bryonolic acid, were also utilized to discriminate signals appearing close to each other, such as those of C-10 to C-13 and C-4 to C-13 of **6**, **16**, **17** and **19**.[3,13)] In order to corroborate the C-10 and C-13 signals of **10**, a lanthanide shift reagent ([Eu(fod)$_3$]) was used.[3)] Assignment of the methyl proton signals was done based on hetero-correlation spectroscopy (^1H–^{13}C COSY) spectral measurement. The results are given in Tables 1 and 2.

Recording nuclear Overhauser effect (NOE) subtraction spectra irradiating 26-, 27-, 28- or 30-methyl protons enabled us to observe the H-18, H-19α spectra of **2**—**21** clearly enough to decide on the experimental *J*-value, *i.e.*, J_{obs} (H-18, 19α), of each compound. When the spectra could not be analyzed as being of the 1st order, the ACD/HNMR spectral simulation program was utilized to identify definite *J*-values (Table 3). The J_{obs} (H-18, 19α) varied widely among **2**—**21**, converging to approximately 1.5—1.7 Hz for **2** and **3** and 13.1—13.3 for **19**—**21**. The values were beyond the smallest value (2.0 Hz) and the largest value (13.0 Hz) possible from Eq. 1 below.

$$J_{(vic)}=7-\cos\phi+5\cos 2\phi \quad (1)$$

$$J_{(vic)}=A-B\cos\phi+C\cos 2\phi \quad (2)$$

in which ϕ is a dihedral angle and parameters corresponding to *A*, *B* and *C* in the Karplus equation (Eq. 2)[14)] were decided empirically to fit the cyclic compound by Bothner-By.[15)] Based on these findings, as well as the steric energy differences described below, we concluded that **2** takes only the F-form, and **20** and **21** only the S-form in $CDCl_3$ solutions.

Conformational studies have frequently utilized the molecular mechanics calculation method.[16)] To test whether the MM2 calculation program would be suitable for our model compounds, the most favorable conformations calculated for

Table 1. (Continued)

Carbon	12	13	14	15	16	17	18	19	20	21
1	34.76	34.78	35.10	34.79	35.08	35.08	34.77	34.77	35.06	34.75
2	24.23	24.26	27.99	24.26	27.97	27.97	24.27	24.26	27.97	24.26
3	80.94	80.97	79.06	81.01	79.07	79.03	81.03	81.01	79.03	80.97
4	37.77	37.78	38.85	37.77	38.84	38.84	37.78	37.77	38.84	37.77
5	50.89	50.93	50.91	51.05	50.97	50.97	51.09	51.08	50.99	51.08
6	19.15	19.15	19.31	19.18	19.32	19.31	19.20	19.18	19.32	19.18
7	27.30	27.31	27.44	27.25	27.39	27.39	27.24	27.20	27.38	27.23
8	135.46	135.49	135.82	136.03	136.11	136.16	136.41	136.41	136.33	136.33
9	133.31	133.34	133.25	133.12	133.13	133.09	132.89	132.89	133.01	132.92
10	37.55	37.56	37.71	37.60	37.75	37.75	37.62	37.61	37.76	37.62
11	20.81	20.81	20.88	20.84	20.85	20.85	20.91	20.88	20.87	20.88
12	30.80	30.75	30.85	30.80	30.94	30.95	30.95	30.95	31.05	31.02
13	37.55	37.62	37.78	37.91	38.08	38.08	38.18	38.12	38.66	38.28
14	40.68	40.67	40.50	40.26	40.15	40.12	40.10	40.02	40.05	40.06
15	26.72	26.78	27.22	27.46	27.68	27.70	27.83	27.86	27.84	27.82
16	36.25	36.33	36.18	35.90	35.81	35.75	35.81	35.68	35.71	35.71
17	31.02	31.16	31.09	31.03	31.00	31.00	30.95	30.85	30.79	30.73
18	43.03	43.04	43.02	42.01	42.06	42.06	42.19	41.86	42.10	41.98
19	29.38	28.80	32.30	28.36	28.88	29.73	30.83	28.08	27.97	27.55
20	31.74	33.17	31.04	35.53	35.93	36.02	33.60	40.25	38.27	39.61
21	29.06	28.91	31.61	26.61	28.20	26.96	29.17	27.19	27.17	27.34
22	37.34	37.57	38.50	38.93	39.93	39.72	40.34	40.52	41.07	41.00
23	28.03	28.04	28.10	28.05	28.12	28.11	28.06	28.05	28.12	28.05
24	16.72	16.73	15.65	16.75	15.66	15.67	16.77	16.76	15.68	16.76
25	19.89	19.88	19.80	19.85	19.78	19.77	19.83	19.81	19.77	19.82
26	25.81	25.90	26.77	27.48	28.02	28.11	28.29	28.46	28.48	28.40
27	18.00	17.92	18.18	17.89	18.06	18.11	18.04	18.05	18.15	17.99
28	31.35	31.34	31.40	31.28	31.23	31.26	31.29	31.21	31.21	31.20
29	73.61	72.80	37.63	110.83	76.97	76.49	39.93	115.88	77.12	89.33
30	27.93	27.59	28.84	23.35	23.73	23.74	24.92	23.97	23.89	23.46
31	171.40		8.27	65.18	17.43	17.45	17.08	65.02	24.87	170.73
32	21.00			65.42			17.37	65.02	25.11	22.64
33								18.87		20.14
34										20.29
3-O<u>C</u>OCH$_3$	170.91	170.97		170.97			170.98	170.97		170.97
3-OCO<u>C</u>H$_3$	21.28	21.30		21.30			21.31	21.30		21.28

a) Data for **1** are not present because of extreme insolubility in $CDCl_3$ (see the text).

Table 2. ^{1}H Chemical Shifts of the Methyl Groups of Bryonolic Acid and Its Derivatives

Methyl proton	1	2	3	4	5	6	7	8	9	10	11
23	0.99	0.87	0.87	0.99	1.00	0.87	1.00	0.99	0.87	0.87	1.00
24	0.78	0.86	0.83	0.80	0.80	0.86	0.80	0.80	0.87	0.87	0.80
25	0.95	0.97	0.94	0.95	0.94	0.97	0.94	0.95	0.97	0.97	0.96
26	0.96	0.96	0.95	0.95	0.94	0.97	0.95	0.99	0.99	1.04	1.06
27	0.88	0.74	0.85	0.75	0.72	0.71	0.68	0.91	0.90	0.86	0.99
28	1.03	1.04	1.03	1.03	1.07	1.05	1.09	1.08	1.08	1.12	1.07
30	1.24	0.94	1.22	1.17	1.04	1.09	1.05	0.98	0.98	1.05	0.96

Methyl proton	12	13	14	15	16	17	18	19	20	21
23	0.88	0.88	1.00	0.88	1.00	1.01	0.89	0.89	1.01	0.89
24	0.88	0.87	0.80	0.88	0.81	0.81	0.88	0.88	0.81	0.88
25	0.98	0.98	0.96	0.98	0.96	0.96	0.98	0.98	0.96	0.99
26	1.11	1.11	1.14	1.17	1.19	1.19	1.19	1.20	1.21	1.21
27	0.93	0.96	0.95	0.95	0.96	0.95	0.94	0.95	0.96	0.95
28	1.12	1.13	1.13	1.15	1.17	1.17	1.16	1.17	1.18	1.18
30	1.00	0.99	0.91	1.01	0.98	0.96	0.90	1.07	1.03	1.03

the S-form of **12** and the F-form for **4** were compared with those elucidated by X-ray crystallographic analyses (Fig. 3).[17] The theoretical and experimental structures were similar with respect to trends and amplitude of distortion and twisting; the distortion forced at the D–E rings of **4** to avoid the severe steric hindrance between the C-27 methyl group

Table 3. S : F Populations **2**—**21** in Equilibrium in $CDCl_3$ Solutions Derived from Calculated Dihedral Angles, ϕ_{calc} (H18–C18–C19–H19α), Observed and Calculated *J*-Values, J_{obs} (H-18, 19α) and J_{calc} (H-18, 19α), and C-26 Chemical Shifts

Compound	Observed *J* values (Hz)	Calculated dihedral angles (degree)[a] and calculated *J* values (Hz)[b]				S : F populations (%) derived from				
		S-form		F-form		J_{obs} (H-18, 19α)			C-26 chemical shift	
	J_{obs}	ϕ_{calc}	J_{calc}	ϕ_{calc}	J_{calc}	P_S (*J*)[c]	P_F (*J*)[d]	Errors (%)[e]	P_S (CS)[c]	P_F (CS)[d]
2	1.5±0.2[f]	176.8±2.0	13.5—13.0	80.0[g]	1.7—1.3	0	100	—	0	100
3	1.7±0.2	176.5±2.0	13.5—13.0	79.2±5.0	2.0—1.1	1	99	±6	0	100
4	2.0±0.2	176.5±2.0	13.5—13.0	79.0±5.0	2.1—1.1	4	96	±6	0	100
5	2.5±0.2[f]	177.3±2.0	13.5—13.0	79.5±5.0	2.0—1.1	8	92	±6	0	100
6	2.7±0.2	176.0±2.0	13.5—13.0	77.7±5.0	2.2—1.2	9	91	±6	4	96
7	2.9±0.2	176.3±2.0	13.5—13.0	75.3±5.0	2.4—1.2	9	91	±7	3	97
8	3.2±0.3	176.4±2.0	13.5—13.0	78.8±5.0	2.1—1.1	14	86	±6	12	88
9	3.2±0.3	176.4±2.0	13.5—13.0	78.8±5.0	2.1—1.1	14	86	±6	12	88
10	5.4±0.3	177.4±2.0	13.5—13.0	73.0±5.0	2.6—1.4	32	68	±5	31	69
11	7.1±0.3	176.8±2.0	13.5—13.0	83.2±5.0	1.8—1.1	48	52	±5	40	60
12	8.6±0.2	176.4±2.0	13.5—13.0	81.9±5.0	1.9—1.1	61	39	±4	58	42
13	9.0±0.2	176.5±2.0	13.5—13.0	83.1±5.0	1.8—1.1	64	36	±5	59	41
14	10.2±0.2	177.4±2.0	13.5—13.0	80.9±5.0	1.9—1.1	74	26	±4	73	27
15	11.8±0.3	176.6±2.0	13.5—13.0	82.9±5.0	1.8—1.1	88	12	±5	85	15
16	12.6±0.3	176.8±2.0	13.5—13.0	81.2±5.0	1.9—1.1	95	5	±5	94	6
17	12.7±0.2	176.8±2.0	13.5—13.0	80.3±5.0	2.0—1.1	95	5	±4	95	5
18	12.9±0.3	177.1±2.0	13.5—13.0	80.2±5.0	2.0—1.1	97	3	±5	98	2
19	13.1±0.3	176.1±2.0	13.5—13.0	82.0±5.0	1.8—1.1	99	1	±5	100	0
20	13.3±0.2	176.0[g]	13.5—13.1	79.7±5.0	2.0—1.1	100	0	—	100	0
21	13.3±0.4	176.4±2.0	13.5—13.0	84.3±5.0	1.7—1.1	100	0	±4	100	0

a) For errors, ±2.0 and ±5.0, see the text. *b*) Equations 3 and 3′ were used to calculate the smallest and largest values, respectively. *c*) Populations of the S-form molecules. The values were calculated using Eq. 5 or 7. The middle values of the upper limit and the smallest side are listed. *d*) Population of the F-form molecules. To calculate the upper limit and the smallest side population, Eq. 4 was used; for the upper limit, conditions *a*=the maximum of the J_{calc} for S-form, *b*=the maximum of the J_{calc} for F-form and *c*=the minimum of the J_{obs}, and for the smallest side, conditions *a*=the minimum of J_{calc} for S-form, *b*=the minimum of the J_{calc} for F-form and *c*=the maximum of the J_{obs} were used. Besides these, conditions *b*=*c* for **2** and *a*=*c* for **20** were used. The middle values of the upper limit and the smallest side are listed. *e*) Differences between the middle values and the upper limit or smallest side of the populations. *f*) Coupling constants (Hz) found in the spectrum measured with a shift reagent at Eu(fod)$_3$-D_{27}/substrate molar ratios of approximately 0.10. *g*) Standards of the calculated dihedral angles: 80.0° for **2** in F-form and 176.0° for **20** in S-form were defined as corresponding to 1.3—1.7 Hz and 13.1—13.5 Hz, respectively.

and the C-29 functional group, and the twisting caused at the D–E rings of **12** to release the duplicated bow–stern interactions owing to its boat–boat conformation, indicated that MM2 calculation could be used in this study. This calculation yielded important information as described below.

Previously, Kikuchi and co-workers claimed that among eight types of conformations possible for the D–E ring system of pachysandiol B (**22**) (Fig. 4), a D:A-friedooleanane class of triterpenes having a common D–E ring system to that of bryonolic acid derivatives, two types, the S-form and F-form, would be far more stable than others based on consideration of steric hindrance by the Dreiding model. Aided by CONFLEX, the MM2 calculation clearly indicated that the occurrence of conformers other than the S-form and F-form in the solutions, if present, would be negligible; they were shown to be less stable by at least 6 kcal/mol than the S-form and F-form conformers.

Differentials of the steric energy between the two conformers [ΔE=E (S-form)−E (F-form)], 3.51 kcal/mol of **2**, −4.02 kcal/mol of **20** and −3.72 kcal/mol of **21**, supported the conclusion described above, that **2**, **20** and **21** take only the F-form or S-form conformations.

According to these conclusions, **2** and **20** were treated as standard, which enabled us to find correspondence of the theoretical dihedral angles, ϕ_{calc} (H18–C18–C19–H19α), to the effective *J*-values between H-18 and H-19α, *i.e.*, 80.0° *vs.* 1.5±0.2 Hz for **2** and 176.0° *vs.* 13.3±0.2 Hz for **20**. These values were used to decide the parameters of Eqs. 3 and 3′; the conditions of 176.0° *vs.* 13.1 Hz and 80.0° *vs.* 1.3 Hz for Eq. 3 and 176.0° *vs.* 13.5 Hz and 80.0° *vs.* 1.7 Hz for 3′ were used.

$$J_{calc}\,(\text{maximum}) = 6.9 - 1.3\cos\phi + 5.3\cos 2\phi \quad (3)$$

$$J_{calc}\,(\text{minimum}) = 6.6 - 1.0\cos\phi + 5.5\cos 2\phi \quad (3')$$

where *A*, *B* and *C* in the Karplus equation were replaced by parameters convenient for our compounds. These equations led us to estimate amount of the error in the S : F populations (see experimental section).

Calculation of the S : F populations was carried out as follows: conformations with the minimal steric energy of three possible rotamers around the C(20)—C(29) bond of both the S-form and F-form conformers of **2**—**21** were found, and the resulting structural data were searched for their dihedral angles, ϕ_{calc} (H18–C18–C19–H19α). They were then converted to the theoretical *J*-values, J_{calc} (H-18, 19α), and the values derived from the rotamer giving the least strain energy among others were used to calculate S : F populations. Calculation of the relative populations was done using Eqs. 4 and 5:

$$\text{population (\%) of F-form molecules} = (a-c)/(a-b)\times 100 \quad (4)$$

$$\text{population (\%) of S-form molecules} = 100 - \text{the population (\%) of F-form molecules.} \quad (5)$$

where "*a*" and "*b*" are theoretical and F-form *J*-values, J_{calc}

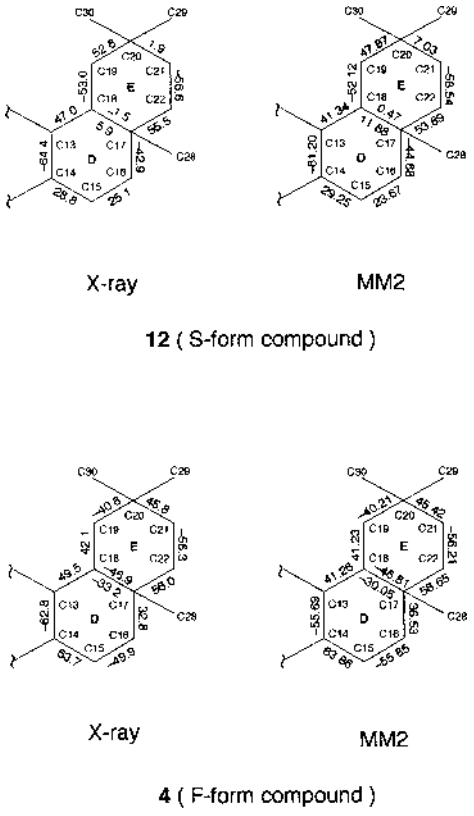

Fig. 3. Comparison of Endocyclic Dihedral Angles (Degree) of the D–E Ring Systems Calculated by MM2 and X-Ray with Respect to the S-Form and F-Form Conformers, **4** and **12**, Respectively

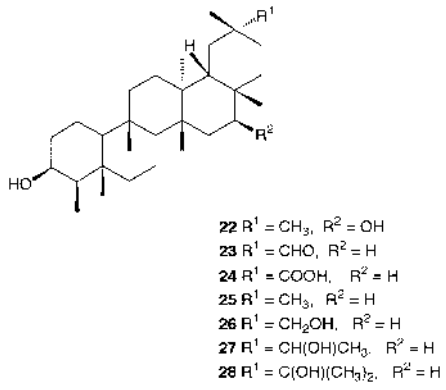

Fig. 4. Structures of D:A-Friedooleananes

(H-18, 19α) of the S-form and F-form conformers in equilibrium, respectively, and "*c*" is an experimental *J*-value, J_{obs} (H-18, 19α), found from the NMR spectra of **2**—**21**.

Although different kinds of functional groups at C-29 may cause different amounts of twisting of the boat–boat structures of the D–E rings in S-form, the calculated dihedral angles, ϕ_{calc} (H18–C18–C19–H19α), of the model compounds in S-form were equivalent to each other with values close to 180° (see Table 3). Studies using a Dreiding model revealed that the D–E rings of the model in boat–boat conformation could freely twist, allowing the dihedral angles in question to remain almost constant at approximately 180°. This led us to consider the dihedral angles of their S-form conformers as practically the same as that of **20** (176.0°). Since the Eqs. 3 and 3′ indicated that small changes in the dihedral angles barely affected *J*-values when the dihedral angles were close to 180°, we took the J_{calc} (H-18, 19α)s of **2**—**19** in S-form as being close to that of **20** and **21**, *i.e.*, 13.3 Hz. As to the F-form, converting two-dimensional C-29 functional groups of **2**—**10** into three-dimensional ones of **11**—**21** led to wider calculated dihedral angles as expected; *e.g.*, **4** (–$COOCH_3$: 79.0) *vs.* **15** (–$CH(=O_2C_2H_4)$: 82.9), **2** (–CHO: 80.0) *vs.* **13** (–CH_2OH: 83.1), **6** (–$COCH_3$: 77.7) *vs.* **16** (–$CH(OH)CH_3$: 81.2) and **8** (–$CH=CH_2$: 78.8) *vs.* **14** (–CH_2CH_3: 80.9). However, replacement of the hydrogen atom at C-29 with a methyl group definitely made the angles narrower, *e.g.*, **5** (–$CH=NNH_2$: 79.5) *vs.* **7** (–$C(=NNH_2)CH_3$: 75.3), **2** (–CHO: 80.0) *vs.* **6** (–$COCH_3$: 77.7), **8** (–$CH=CH_2$: 78.8) *vs.* **10** (–$C(=CH_2)CH_3$: 73.0), **11** (–CH_3: 83.2) *vs.* **14** (–CH_2CH_3: 80.9) *vs.* **18** (–$CH(CH_3)_2$: 80.2), **13** (–CH_2OH: 83.1) *vs.* **17** (–$CH(OH)CH_3$: 80.3) *vs.* **20** (–$C(OH)(CH_3)_2$: 79.7) and **15** (–$CH(=O_2C_2H_4)$: 82.9) *vs.* **19** (–$C(CH_3)(=O_2C_2H_4)$: 82.0). These results suggest that in amplitude, the dihedral angles in question do not simply increase in parallel with the bulkiness of the C-29 functional groups. Taking these findings into account, errors of the angles calculated were estimated within plus or minus 2° for the S-form and 5° for the F-form (see Table 3).[18)]

Besides the J_{obs} (H-18, 19α), chemical shifts of several nuclei around the D-ring also varied significantly among the model compounds, **2**—**21** (see Tables 1, 2). Because these nuclei are far away from the C-29 functional groups, the shift data were expected to offer additional information on the S : F populations. Structural data from the MM2 calculation indicated that changes in the conformations at the D–E ring moieties due to changes in the functional groups at C-29 are extremely small in the S-form conformers, but significant in the F-form conformers. The changes are most likely to occur at the C-19—C-21 of their E-rings, but those around the D-rings are minor (see Fig. 5). This led us to assume that chemical shifts of the nuclei around the D-rings proper to both conformers of the model compounds are identical to those of **2** and **20**; *e.g.*, 22.33 ppm to the C-26 of the F-form conformers and 28.48 ppm to those of the S-form conformers. According to these, the S : F populations were calculated from the C-26 shift data by Eqs. 6 and 7 in comparison with those from the *J*-values.

$$\text{population (\%) of F-form molecules} = (28.5 - d)/(28.5 - 22.3) \times 100 \quad (6)$$

$$\text{population (\%) of S-form molecules} = 100 - \text{the population (\%) of F-form molecules} \quad (7)$$

where "*d*" is the observed C-26 chemical shift.

Calculation from other nuclei shift data, *i.e.*, those of carbons C-8 and C-15 and methyl protons at C-26, which varied significantly among the model compounds, were also carried out. The results agree well with that from C-26, supporting the conclusion described above. The C-26 methyl proton chemical shifts (0.96 ppm) of **1**, which prevented us from determining both its J_{obs} (H-18, 19α) and C-26 chemical shift because of the extreme insolubility in $CDCl_3$, indicated that **1** took solely the F-form conformation, as did **2**.

Discussion and Conclusion

Relative populations calculated from independent spectral data, *i.e.*, *J*-values and chemical shifts, are consistent with

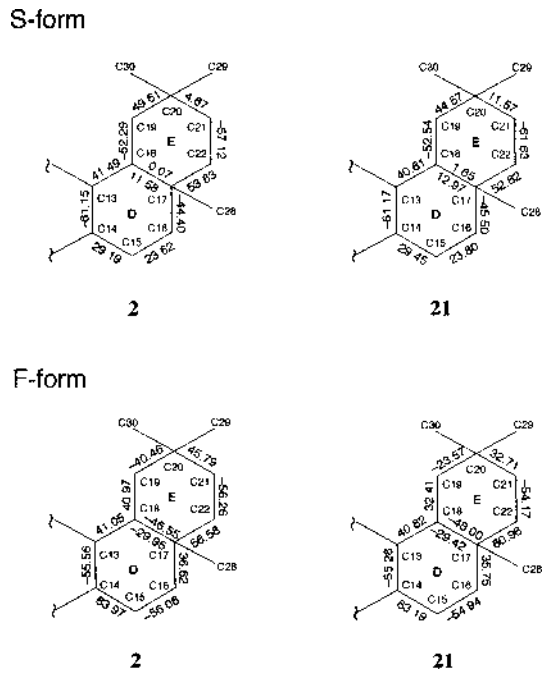

Fig. 5. Endocyclic Dihedral Angles (Degree) of the D–E Ring Systems Calculated by MM2 with Respect to the S-Form and F-Form Conformers, **2** and **21**, Respectively

each other, indicating that the results are reliable. Studying the conformational situation of the D–E ring moiety of bryonolic acid derivatives and then calculating MM2 enabled us to evaluate the reliability of the S : F populations derived from the *J*-values, offering much more detailed information on the steric features of bryonolic acid derivatives than those previously reported.[3)]

The principal factor determining the S : F populations was whether the functional groups at C-29 are trigonal or tetrahedral: the typical values of populations of the "trigonal group," **2**—**10**, were 0 : 100—32 : 68, and those of the "tetrahedral group," **11**—**21**, were 48 : 52—100 : 0. For the "trigonal group," substituting a methyl group for a hydrogen atom on the C-29 functional groups of **2** and **9** led to an increase in S-form conformers by 9 and 18. For the "tetrahedral group," the tendency toward S-form conformations increased stepwise as the number of substituents linking to C-29 increased; S : F populations of the "methyl group" **11**, were 48 : 52; "monosubstituted group" **12**—**14**, 61 : 39—74 : 26; "disubstituted group" **15**—**18**, 88 : 12—97 : 3; and the "trisubstituted group" **19**—**21**, 99 : 1—100 : 0. These results serve as an index for predicting the S : F populations of new compounds with functional groups at C-29. Steric difference at the C-29 of anomeric **16** and **17** little affected their S : F populations.

The significant difference between the C-26 chemical shifts of the S-form and F-form conformers may be interpreted as arising from the γ-gauche effect. In addition to three protons, H-7β, H-12β and H-18, located at the γ-gauche positions of the 26-methyl group, the F-form conformer has an additional γ-gauche proton, H-16β, which may cause significant upfield C-26 chemical shifts of the F-form conformers.

Bryonolic acid was found to have a unique character, giving derivatives with S : F populations of 0 : 100—100 : 0. Although Friedelin derivatives (**23**—**28**), D:A-friedooleanane class of triterpenes (Fig. 4), have structures analogous to bryonolic acid derivatives at the C–E ring moiety, MM2 calculation suggested that it tended to have more S-form conformers than the bryonolic acid derivatives; the steric energy difference between the S-form and F-form conformers, [$\Delta E = E$ (S-form) $- E$ (F-form)] calculated for these compound were 1.9, 2.0, 0.0, −0.17, −1.15 and −5.0 kcal/mol, respectively. These differences were significantly smaller than 3.5, 3.4, 1.3, 0.28, −0.05 and −4.0 kcal/mol compared with those of the bryonolic acid derivatives **2**, **3**, **11**, **13**, **16** and **20** corresponding to **23**—**28**, suggesting that Friedelin derivatives could not solely adopt the F-form in solutions.

This study has shown that by selecting appropriate functional groups at C-29, various types of bryonolic acid derivatives can be designed with respect to relative populations of the S-form and F-form conformers. We suggest that bryonolic acid is an excellent starting material for preparing a series of experimental compounds which vary from each other stepwise over wide ranges with respect to relative populations between two components in solution in equilibrium.

Experimental

The melting points were measured using a Yanagimoto micromelting point apparatus and were not corrected. Optical rotations were measured at room temperature with a JASCO P-1020 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL GSX-500 spectrometer operating at 500 MHz for protons and 125 MHz for carbon. Homo decoupling for **2**—**7** and NOE experiments were carried out on the JEOL ECP-500 spectrometer operating at 500 MHz for protons. Spectra were recorded on 50—30 mg ml^{-1} concentration solutions with a probe temperature of 35 °C in $CDCl_3$, and referred to tetramethylsilane (TMS). Eu(fod)$_3$-D$_{27}$ for Lanthanide-induced shift measurements were purchased from E. Merck, and no further purification or drying of the reagents was attempted before use. Typical conditions for proton spectral measurements were 40—120 transients and a spectral width of 4000 Hz with 32 K data points. Proton-decoupled carbon spectra were obtained under typical conditions: 5000—10000 transients, 21 μs pulse delay and a spectral width of 32000 Hz with 64 K data points. In the case of the homo decoupling spectra, 6000 transients, a spectral width of 7500 Hz with 64 K data points and zero filled to 256 K to give a digital resolution of 0.025 Hz. NOE conditions were 6000 transients, a spectral width of 7500 Hz with 16 K data points and zero filled to 64 K to give a digital resolution of 0.1 Hz. Two-dimensional (2D) NMR spectra (^{1}H–^{13}C COSY, ^{1}H–^{1}H COSY and HMBC) were performed on the JEOL GSX-500 spectrometer using standard JEOL CHSHF, DQFN and HMBC pulse sequences. MS and HR-MS were taken on a JEOL JMS DX-300 mass spectrometer. Unless otherwise specified, MS spectra were recorded under electron impact (EI) at 70 eV. ACD/HNMR predictor was purchased from Advanced Chemistry Development, Inc. The conformational analysis was carried out using CAChe WorkSystem version 4.1 software (Fujitsu Co., Ltd.). For every compound (**2**—**21**), rotational analysis was performed with incremental changes in the dihedral angle C19–C20–C29–X (X=H, alkyl, OH or OAc) or C19–C20–C29=X (X=O or N) in 2 steps from 60° to −180° to CONFLEX.

Bryonolic acid was isolated as crystals from the $CHCl_3$ extract of cultured cells of *Luffa cylindrica*, and its derivatives, **3**, **4**, **12** and **13** were prepared by reported methods.[3)]

3: mp 221—222 °C. $[\alpha]_D$ 5.4° (c=1.0, $CHCl_3$). EI-MS *m/z*: 440 (M^+), 425 (M^+-CH_3), 395 (M^+-COOH), 243 ($C_{18}H_{27}^+$ containing A, B and C rings), 235 ($C_{15}H_{23}O_2^+$ containing D and E rings), 231 ($C_{17}H_{27}^+$ containing A and B rings, base peak), 191. High resolution (HR)-EI-MS *m/z*: 440.3649 (Calcd for $C_{30}H_{48}O_2$: 440.3654).

4: mp 196—197 °C. $[\alpha]_D$ 9.8° (c=1.0, $CHCl_3$). EI-MS *m/z*: 470 (M^+, base peak), 455 (M^+-CH_3), 452 (M^+-H_2O), 437 ($M^+-CH_3-H_2O$), 411 ($M^+-COOCH_3$), 259 ($C_{18}H_{27}O^+$ containing A, B and C rings), 249 ($C_{16}H_{25}O_2^+$ containing D and E rings), 247 ($C_{17}H_{27}O^+$ containing A and B rings), 189. HR-EI-MS *m/z*: 470.3759 (Calcd for $C_{31}H_{50}O_3$: 470.3760).

12: mp 212—213 °C. $[\alpha]_D$ 24.3° (c=1.0, $CHCl_3$). EI-MS *m/z*: 526 (M^+), 511 (M^+-CH_3), 466 (M^+-CH_3COOH), 453 ($M^+-CH_2OCOCH_3$), 301 ($C_{20}H_{29}O_2^+$ containing A, B and C rings), 289 ($C_{19}H_{29}O_2^+$ containing A and B

rings), 263 ($C_{17}H_{27}O_2^+$ containing D and E rings), 203 (base peak), 189. HR-EI-MS *m/z*: 526.4019 (Calcd for $C_{34}H_{54}O_4$: 526.4022).

13: mp 245—246 °C. $[\alpha]_D$ 34.0° (*c*=1.0, $CHCl_3$). EI-MS *m/z*: 484 (M^+), 469 (M^+-CH_3), 453 (M^+-CH_2OH, base peak), 424 (M^+-CH_3COOH), 301 ($C_{20}H_{29}O_2^+$ containing A, B and C rings), 289 ($C_{19}H_{29}O_2^+$ containing A and B rings), 221 ($C_{15}H_{25}O^+$ containing D and E rings), 189. HR-EI-MS *m/z*: 484.3908 (Calcd for $C_{32}H_{52}O_3$: 484.3916).

Compound 2 Chromium(VI) oxide (2.2 g, 22 mmol) was added stepwise to a solution of **13** (630 mg, 1.2 mmol) in pyridine (22 g, 178 mmol) under stirring and ice-cooling, and the mixture was stirred for another 1 h at room temperature. The reaction mixture was poured into 5% HCl containing ice, then shaken with benzene. The combined organic layer was washed with H_2O and evaporated to give a residue, which was purified by recrystallization from methanol. mp 216—217 °C. $[\alpha]_D$ 25.7° (*c*=1.0, $CHCl_3$). EI-MS *m/z*: 482 (M^+, base peak), 467 (M^+-CH_3), 453 (M^+-CHO), 422 (M^+-CH_3COOH), 301 ($C_{20}H_{29}O_2^+$ containing A, B and C rings), 289 ($C_{19}H_{29}O_2^+$ containing A and B rings), 219 ($C_{15}H_{23}O^+$ containing D and E rings), 189. HR-EI-MS *m/z*: 482.3753 (Calcd for $C_{32}H_{50}O_3$: 482.3760).

Compound 5 A solution of a mixture of **2** (20 mg, 0.04 mmol), diethylene glycol (10 ml), hydrazine hydrate (3 ml) and potassium hydroxide (1 g) in a mixture of benzene (4 ml) and methanol (10 ml) was heated in a water bath for 2 h at 60 °C. The reaction mixture was poured into 5% HCl containing ice and shaken with benzene. The combined organic layer was washed with H_2O and evaporated to give a residue, which was purified by recrystallization from methanol. mp 241—243 °C. $[\alpha]_D$ 31.7° (*c*=0.8, $CHCl_3$). Positive-ion FAB-MS (3-nitrobenzyl alcohol) *m/z*: 455 (M^++1), 438 (M^+-NH_2), 436 (M^+-H_2O), 421 ($M^+-CH_3-H_2O$), 259 ($C_{18}H_{27}O^+$ containing A, B and C rings), 247 ($C_{17}H_{27}O^+$ containing A and B rings), 189. HR-EI-MS *m/z*: 454.3930 (Calcd for $C_{30}H_{50}N_2O$: 454.3923).

Compound 6 Chromium(VI) oxide (1 g, 10 mmol) was added stepwise to a solution of 3-acetyl-29-methyl bryonyl alcohol (70 mg, 0.14 mmol) in pyridine (10 g, 126 mmol) under stirring and ice-cooling, and the mixture was stirred for another 4 h at room temperature. The reaction mixture was treated as descried for **2** above to give pure **6**. mp 196—197 °C. $[\alpha]_D$ 45.8° (*c*=0.3, $CHCl_3$). EI-MS *m/z*: 496 (M^+), 481 (M^+-CH_3), 453 (M^+-COCH_3), 436 (M^+-CH_3COOH), 301 ($C_{20}H_{29}O_2^+$ containing A, B and C rings), 289 ($C_{19}H_{29}O_2^+$ containing A and B rings), 233 ($C_{16}H_{25}O^+$ containing D and E rings), 189, 44 (base peak). HR-EI-MS *m/z*: 496.3915 (Calcd for $C_{33}H_{52}O_3$: 496.3916).

Compound 7 A solution of a mixture of **6** (20 mg, 0.04 mmol), diethylene glycol (10 ml), hydrazine hydrate (2 ml) and potassium hydroxide (0.5 g) in a mixture of benzene (4 ml) and methanol (10 ml) was heated for 2 h at 50 °C. The reaction mixture was treated as described above to give pure **7**. mp 211—212 °C. $[\alpha]_D$ 79.6° (*c*=0.4, $CHCl_3$). EI-MS *m/z*: 468 (M^+), 453 (M^+-CH_3), 452 (M^+-NH_2), 450 (M^+-H_2O), 435 ($M^+-CH_3-H_2O$), 259 ($C_{18}H_{27}O^+$ containing A, B and C rings), 247 ($C_{16}H_{27}N_2^+$ containing D and E rings and $C_{17}H_{27}O^+$ containing A and B rings), 189, 44 (base peak). HR-EI-MS *m/z*: 468.4079 (Calcd for $C_{31}H_{52}N_2O$: 468.4079).

Compound 8 According to the Cainelli procedure[19] based on the Wittig reaction, under an argon atmosphere, methylene iodide (66 mg, 0.24 mmol) in Et_2O was added to a solution of **2** (100 mg, 0.22 mmol) and magnesium amalgam (8 g) in Et_2O, and the entire mixture was stirred under reflux for 3 h. The reaction mixture was poured into 5% HCl and shaken with benzene. The combined organic layer was washed with H_2O and evaporated to obtain the residue. This residue was purified by HPLC and then recrystallized from methanol. mp 168—169 °C. $[\alpha]_D$ 20.0° (*c*=0.1, $CHCl_3$). EI-MS *m/z*: 438 (M^+, base peak), 423 (M^+-CH_3), 420 (M^+-H_2O), 405 ($M^+-CH_3-H_2O$), 259 ($C_{18}H_{27}O^+$ containing A, B and C rings), 247 ($C_{17}H_{27}O^+$ containing A and B rings), 217 ($C_{16}H_{25}^+$ containing D and E rings), 189. HR-EI-MS *m/z*: 438.3861 (Calcd for $C_{31}H_{50}O$: 438.3862).

Compound 9 This compound was prepared by acetylation of **8** with acetic anhydride in pyridine, followed by recrystallization from methanol. mp 181—182 °C. $[\alpha]_D$ 18.2° (*c*=0.5, $CHCl_3$). EI-MS *m/z*: 480 (M^+, base peak), 465 (M^+-CH_3), 453 ($M^+-CH=CH_2$), 420 (M^+-CH_3COOH), 301 ($C_{20}H_{29}O_2^+$ containing A, B and C rings), 289 ($C_{19}H_{29}O_2^+$ containing A and B rings), 217 ($C_{16}H_{25}^+$ containing D and E rings), 189. HR-EI-MS *m/z*: 480.3961 (Calcd for $C_{33}H_{52}O_2$: 480.3967).

Compound 10 and 21 Acetylation of **20** with acetic anhydride in pyridine gave a mixture of **10** and **21**. After treatment in the usual manner, these compounds were isolated by HPLC, then purified by recrystallization from methanol.

10: mp 219—220 °C. $[\alpha]_D$ 11.2° (*c*=1.0, $CHCl_3$). EI-MS *m/z*: 494 (M^+), 479 (M^+-CH_3), 453 [$M^+-C(=CH_2)CH_3$], 434 (M^+-CH_3COOH), 301 ($C_{20}H_{29}O_2^+$ containing A, B and C rings), 289 ($C_{19}H_{29}O_2^+$ containing A and B rings), 231 ($C_{17}H_{27}^+$ containing D and E rings, base peak), 189. HR-EI-MS *m/z*: 494.4124 (Calcd for $C_{34}H_{54}O_2$: 494.4124).

21: mp 194—195 °C. $[\alpha]_D$ 38.9° (*c*=1.0, $CHCl_3$). EI-MS *m/z*: 554 (M^+), 539 (M^+-CH_3), 494 (M^+-CH_3COOH), 434 (M^+-2CH_3COOH), 301 ($C_{20}H_{29}O_2^+$ containing A, B and C rings), 289 ($C_{19}H_{29}O_2^+$ containing A and B rings), 231 ($C_{19}H_{31}O_2^+$ containing D and E rings$-CH_3COOH$, base peak), 189. HR-EI-MS *m/z*: 554.4332 (Calcd for $C_{36}H_{58}O_2$: 554.4335).

Compound 11 A solution of a mixture of **2** (20 mg, 0.04 mmol), diethylene glycol (50 ml), hydrazine hydrate (2 ml) and potassium hydroxide (3 g) in a mixture of benzene (5 ml) and methanol (10 ml) was heated for 4 h at 190 °C. The reaction mixture was poured into 5% HCl containing ice and shaken with benzene. The combined organic layer was washed with H_2O and evaporated to give a residue, which was purified by recrystallization from methanol. mp 185—186 °C. $[\alpha]_D$ 28.2° (*c*=0.7, $CHCl_3$). EI-MS *m/z*: 426 (M^+), 411 (M^+-CH_3), 393 ($M^+-CH_3-H_2O$), 259 ($C_{18}H_{27}O^+$ containing A, B and C rings), 247 ($C_{17}H_{27}O^+$ containing A and B rings), 205 ($C_{15}H_{25}^+$ containing D and E rings), 189, 44 (base peak). HR-EI-MS *m/z*: 426.3859 (Calcd for $C_{30}H_{50}O$: 426.3862).

Compound 14 A solution of a mixture of **6** (20 mg, 0.04 mmol), diethylene glycol (50 ml), hydrazine hydrate (2 ml) and potassium hydroxide (3 g) in a mixture of benzene (5 ml) and methanol (10 ml) was heated for 4 h at 190 °C. The reaction mixture was treated as described above to give pure **14**. mp 192—193 °C. $[\alpha]_D$ 45.0° (*c*=0.7, $CHCl_3$). EI-MS *m/z*: 440 (M^+), 425 (M^+-CH_3), 411 ($M^+-CH_2CH_3$), 407 ($M^+-CH_3-H_2O$), 259 ($C_{18}H_{27}O^+$ containing A, B and C rings), 247 ($C_{17}H_{27}O^+$ containing A and B rings), 219 ($C_{16}H_{27}^+$ containing D and E rings, base peak), 189. HR-EI-MS *m/z*: 440.4021 (Calcd for $C_{31}H_{52}O$: 440.4018).

Compound 15 A solution of a mixture of **2** (12 mg, 0.025 mmol), *p*-TsOH·H_2O (2 mg, 0.01 mmol) and ethylene glycol (100 mg, 1.61 mmol) in benzene was refluxed for 10 h. The reaction mixture was poured into saturated aqueous $NaHCO_3$ solution and shaken with benzene. The combined organic layer was washed with H_2O and evaporated to give a residue, which was purified by recrystallization from methanol. mp 238—239 °C. $[\alpha]_D$ 42.0° (*c*=0.5, $CHCl_3$). EI-MS *m/z*: 526 (M^+), 511 (M^+-CH_3), 466 (M^+-CH_3COOH), 453 ($M^+-C_3H_5O_2^+$; 1,3-dioxolan-2-yl), 301 ($C_{20}H_{29}O_2^+$ containing A, B and C rings), 289 ($C_{19}H_{29}O_2^+$ containing A and B rings), 263 ($C_{17}H_{27}O_2^+$ containing D and E rings), 189, 73 ($C_3H_5O_2^+$; 1,3-dioxolan-2-yl, base peak). HR-EI-MS *m/z*: 526.4024 (Calcd for $C_{34}H_{56}O_4$: 526.4022).

Compound 16, 17 and 3-Acetyl-29-methyl Bryonyl Alcohol A solution of MeMgI in Et_2O, prepared from Mg (144 mg, 6.0 mmol) and MeI (855 mg, 6.0 mmol), was added dropwise to a solution of **2** (100 mg, 0.21 mmol) in Et_2O under gentle reflux. After the mixture was stirred for 1 h at room temperature, the reaction was quenched with H_2O. The reaction mixture was poured into 5% HCl and shaken with benzene. The combined organic layer was washed with H_2O and evaporated to give a residue. **16** and **17** were isolated by silica gel column chromatographic treatment and then recrystallized from methanol.

16: mp 232—233 °C. $[\alpha]_D$ 49.4° (*c*=0.2, $CHCl_3$). EI-MS *m/z*: 456 (M^+), 441 (M^+-CH_3), 438 (M^+-H_2O), 423 ($M^+-CH_3-H_2O$), 411 [$M^+-CH(OH)CH_3$, base peak], 259 ($C_{18}H_{27}O^+$ containing A, B and C rings), 247 ($C_{17}H_{27}O^+$ containing A and B rings), 235 ($C_{16}H_{27}O^+$ containing D and E rings), 189. HR-EI-MS *m/z*: 456.3970 (Calcd for $C_{31}H_{52}O_2$: 456.3967).

17: mp 232—233 °C. $[\alpha]_D$ 48.7° (*c*=0.6, $CHCl_3$). EI-MS *m/z*: 456 (M^+), 441 (M^+-CH_3), 438 (M^+-H_2O), 423 ($M^+-CH_3-H_2O$), 411 [$M^+-CH(OH)CH_3$, base peak], 259 ($C_{18}H_{27}O^+$ containing A, B and C rings), 247 ($C_{17}H_{27}O^+$ containing A and B rings), 235 ($C_{16}H_{27}O^+$ containing D and E rings), 189. HR-EI-MS *m/z*: 456.3974 (Calcd for $C_{31}H_{52}O_2$: 456.3967).

Compound 18 A suspension of a mixture of **10** (10 mg, 0.02 mmol) and PtO_2 (3 mg) in methanol was stirred at room temperature under H_2 atmosphere for 2 h. The PtO_2 catalyst was filtered off and the filtrate was evaporated to give a residue, then the residue was recrystallized from methanol to give pure **18**. mp 265—267 °C. $[\alpha]_D$ 50.5° (*c*=0.5, $CHCl_3$). EI-MS *m/z*: 496 (M^+), 481 (M^+-CH_3), 453 [$M^+-CH(CH_3)_2$, base peak], 436 (M^+-CH_3COOH), 301 ($C_{20}H_{29}O_2^+$ containing A, B and C rings), 289 ($C_{19}H_{29}O_2^+$ containing A and B rings), 233 ($C_{17}H_{29}^+$ containing D and E rings), 189. HR-EI-MS *m/z*: 496.4285 (Calcd for $C_{34}H_{56}O_4$: 496.4280).

Compound 19 A solution of a mixture of **6** (10 mg, 0.02 mmol), *p*-TsOH·H_2O (2 mg, 0.01 mmol) and ethylene glycol (100 mg, 1.61 mmol) in benzene was refluxed for 16 h. The reaction mixture was treated as described above to give pure **19**. mp 267—268 °C. $[\alpha]_D$ 50.5° (*c*=0.5, $CHCl_3$). EI-MS *m/z*: 540 (M^+), 525 (M^+-CH_3), 480 (M^+-CH_3COOH), 454 ($M^+-C_4H_7O_2^+$; 2-methyl-1,3-dioxolan-2-yl), 301 ($C_{20}H_{29}O_2^+$ containing A, B and C rings), 289 ($C_{19}H_{29}O_2^+$ containing A and B rings), 189, 87 ($C_4H_7O_2^+$; 2-methyl-1,3-dioxolan-2-yl, base peak). HR-EI-MS *m/z*: 540.4175 (Calcd for

$C_{35}H_{56}O_4$: 540.4178).

Compound 20 A solution of MeMgI in Et_2O, prepared from Mg (2 g, 83.0 mmol) and MeI (12 g, 84.0 mmol), was added dropwise to a solution of **4** (200 mg, 0.43 mmol) in Et_2O under gentle reflux. The mixture was then treated as described for **16** and **17** to give pure **20**. mp 260—261 °C. $[\alpha]_D$ 53.0° (c=0.6, $CHCl_3$). EI-MS m/z: 470 (M^+), 455 (M^+-CH_3), 452 (M^+-H_2O), 437 ($M^+-CH_3-H_2O$), 411 [$M^+-C(OH)(CH_3)_2$], 259 ($C_{18}H_{27}O^+$ containing A, B and C rings, base peak), 247 ($C_{17}H_{27}O^+$ containing A and B rings), 231 ($C_{17}H_{29}O^+$ containing D and E rings$-H_2O$), 189. HR-EI-MS m/z: 470.4129 (Calcd for $C_{32}H_{51}O_2$: 470.4124).

Preparation of ^{13}C-Enriched Bryonolic Acid Fifteen days after the inoculation of *L. cylindrica* cells, 300 mg of sodium [2-^{13}C]acetate was added to 500 ml flasks containing cells growing in 300 ml of suspension medium (Linsmaier–Skoog medium supplemented with 3% glucose and 10^{-7} M 2,4-D). After a subsequent incubation period of 6—7 d, the cells were harvested. Dried and powdered cells were extracted with $CHCl_3$; the $CHCl_3$ solution was then condensed to yield fine needles of ^{13}C-enriched bryonolic acid. Recrystallization of the material from $CHCl_3$ gave 100 mg (from eight flasks).

Preparation of ^{13}C-Enriched Derivatives 2-^{13}C-enriched species, **10**, **16**, **17** and **19**, were prepared in the same manner as the unlabeled compounds.

Equations 3 and 3′ Parameters A, B and C for Eqs. 3 and 3′ were determined to fit the following conditions:

For Eq. 3

$$1.7\ (\mathrm{Hz}) = A - B\cos(80.0) + C\cos(80.0\times 2)$$

$$8.2\ (\mathrm{Hz}) = A - B\cos(32.1) + C\cos(32.1\times 2)$$

$$13.5\ (\mathrm{Hz}) = A - B\cos(176.0) + C\cos(176.0\times 2)$$

and for Eq. 3′

$$1.3\ (\mathrm{Hz}) = A - B\cos(80.0) + C\cos(80.0\times 2)$$

$$8.2\ (\mathrm{Hz}) = A - B\cos(32.1) + C\cos(32.1\times 2)$$

$$13.1\ (\mathrm{Hz}) = A - B\cos(176.0) + C\cos(176.0\times 2)$$

Equations 3 and 3′ were used to determine the maximum and minimum theoretical J_{calc} (H-18, 19α) of the S-form and F-form molecules for **2**—**21**. The values 8.2 (Hz) and 32.1 given in the above equations came from the observed J-value between H-18 and H-19β and the calculated dihedral angle for the H18–C18–C19–H19β, respectively.

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References and Notes

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