Structure and Conformation of Erythronolide B and (8*S*)-8-Fluoroerythronolide B Spiroacetals and of Their Acetyl Derivatives. A ^{1}H NMR Study

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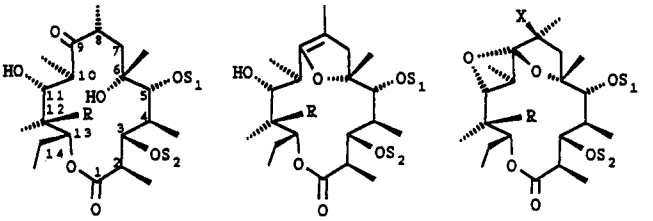
The spiroacetal derivatives of Erythronolide B and of (8*S*)-8-fluoroerythronolide B are shown to hold the 5,9;6,9-spiroacetal structures **14** and **13**, respectively. The conformational properties of the 12-membered macrolide ring are highly dependent on the substituent at C(8). The 8-fluoro spiroacetal **13** shows an endocyclic orientation of the Me(4) and H(12) groups. In the case of the unsubstituted spiroacetal **14** the protons H(3), H(2), and H(11) point toward the inner part of the molecule. For the diacetyl derivatives **15** and **16** the groups Me(4), H(11), and H(2) are oriented inside and the two acetyl substitutents outside the macrolide ring.

The naturally occurring erythromycins A **1** and B **2**, representative members of the macrolide class of antibiotics, are composed of a polysubstituted 14-membered lactone ring linked to the desosamine and cladinose sugar units. **1** and **2** are known to form hemiacetal and spiroacetal derivatives in acidic media as main products.[1-4] The aglycons of the erythromycins A and B, the erythronolides A **3** and B **4**, behave similarly and their reactions with electrophilic agents were extensively investigated. Thus, treatment of **3** and **4** with glacial acetic acid gave a 9:1 mixture of the 8,9-anhydroerythronolides A **5** and B **8** and of the 6,9;9,11-spiroacetal derivatives **7** and **8**.[5] In analogy with the behavior of the erythromycin A, the hydrolysis of **5** and **7** with methanolic HCl gave the erythronolide 6,9;9,12-spiroacetal A **11**.[5] The reaction of **3** and **4** with fluorinating agents such CF_3OF gave as main products the (8*S*)-8-fluoroerythronolides 6,9;9,11-spiroacetals A **9** and B **10**.[6] In contrast with these findings we have found recently[7] that the 8,9-anhydroerythronolide B **6** was converted quantitatively with *N*-bromoacetamide to (8*S*)-8-bromoerythronolide 6,9;5,9-spiroacetal B **12**, the first example of a new class of spiroacetal derivatives.

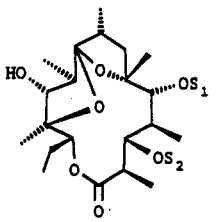
In this paper, we present the results of a ^{1}H NMR study carried out on the spiroacetals of erythronolide B and of (8*S*)-8-fluoroerythronolide B, previously reported as 5,9;9,11-spiroacetals.[5,6] We show that their structures are the 5,9;6,9-spiroacetals **13** and **14**. Moreover, particular emphasis is laid to the conformational properties of these rings, which are highly dependent on the substituent at C(8). In addition, the diacetyl derivatives, **15** and **16** have also been examined to gain more information about the driving forces responsible for the conformational variety of these rings.



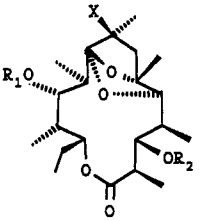
1 R = OH, S1 = desosaminyl, S2 = cladinosyl.
2 R = H, S1 = desosaminyl, S2 = cladinosyl.
3 R = OH, S1 = S2 = H
4 R = H, S1 = S2 = H

5 R = OH, S1 = S2 = H
6 R = H, S1 = S2 = H

7 R = OH, X = H, S1 = S2 = H
8 R = H, X = H, S1 = S2 = H
9 R = OH, X = F, S1 = S2 = H
10 R = H, X = F, S1 = S2 = H



11 S1 = S2 = H



12 X = Br, R1 = R2 = H
13 X = F, R1 = R2 = H
14 X = H, R1 = R2 = H
15 X = F, R1 = R2 = Ac
16 X = H, R1 = R2 = Ac

Results and Discussion

Structure Determination. Full assignment of the proton NMR spectra of compounds **13-16** was achieved by 2D techniques. As an example of the strategy followed for the proton assignment the contour plot of the COSY experiment performed on the diacetyl derivative **16** is reported in Figure 1. Protons belonging to the fragment C(10(-C(11)-C(12)-C(13) were distinguished using H(13) as starting point, clearly recognizable by its low field resonance and its multiplicity (ABXY spin system). The correlation pattern can be easily followed (Figure 1) leading to the unambigous identification of protons H(12), H(13), and H(10). Similarly, within the fragment C(2)-C(3)-C(4)-C(5), H(2) can be recognized by its multiplicity (A_3XY spin system), and the chemical shifts of the protons H(3), H(4), and H(5) can be identified from their correlation map.

The chemical shifts and coupling constants of compounds **13-16** are collected in Tables I and II, respectively.

(1) Kurath, P.; Jones, P. H.; Egan, R. S.; Perun, T. J. *Experientia* **1971**, *27*, 362.
(2) Krowicki, K.; Zamojski, A. *J. Antibiot.* **1973**, *26*, 582; *Ibid.* **1973**, *26*, 582.
(3) Kurath, P.; Martin, J. R.; Tadanier, J.; Goldstein, A. W.; Egan, R. S.; Dunnigan, D. A. *Helv. Chim. Acta* **1973**, *56*, 1557.
(4) Egan, R. S.; Martin, J. R.; McAlpine, J. B.; Kurath, P.; Stanaszek, R. S., Goldstein, A. W.; Johnson, L. F. *J. Antibiot.* **1978**, *31*, 55.
(5) Toscano, L.; Seghetti, E.; Inglesi, M.; Fioriello, G. *Gazz. Chim. Ital.* **1984**, *114*, 173.
(6) Toscano, L.; Fioriello, G.; Silingardi, S.; Inglesi, M. *Tetrahedron* **1984**, *40*, 2177.
(7) Auricchio, S.; Fronza, G.; Meille, S. V.; Mele, A.; Favara, D. *J. Org. Chem.* **1991**, *56*, 2250.

0022-3263/92/1957-0452$03.00/0

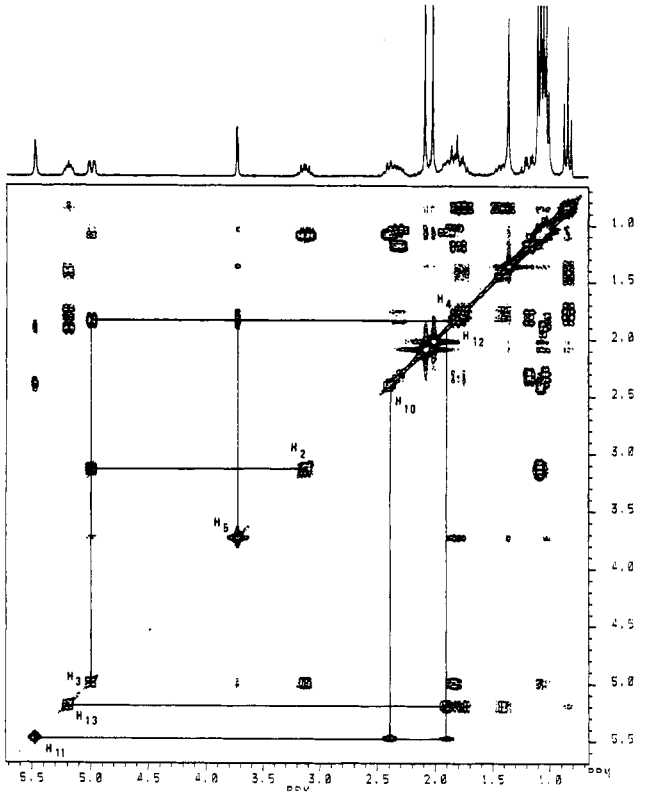


Figure 1. Long-range $^1H,^1H$ COSY spectrum, obtained with a delay period of 0.07 s, of the 3,11-di(*O*-acetyl)erythronolide 5,9;6,9-spiroacetal B **16**. The solid lines indicate the connectivities of the protons belonging to the fragments C(2)–C(5) and C(10)–C(13).

Table I. ^1H Chemical Shifts of Compounds 13–16[a]

	13[b]	14[c]	15[d]	16[e]
H-2	2.50 (2.52)	2.59	2.92	3.13
H-3	3.76 (3.53)	4.59	5.03	5.00
H-4	1.95 (1.47)	1.98	1.83	1.85
H-5	3.78 (3.64)	3.57	3.60	3.73
H-7a	1.98 (1.73)	1.81	1.83	1.80
H-7b	1.78 (1.23)	1.20	1.83	1.18
H-8		2.33		2.32
H-10	2.90 (3.14)	2.07	2.69	2.39
H-11	3.65 (3.59)	4.18	5.44	5.48
H-12	2.50 (2.67)	1.72	1.85	1.90
H-13	4.90 (5.12)	5.27	5.09	5.19
H-14a	2.19 (2.52)	1.72	1.75	1.75
H-14b	1.76 (1.63)	1.51	1.43	1.44
Me-2	1.30 (1.29)	1.18	1.13	1.09
Me-4	0.93 (1.06)	1.05	1.12	1.02
Me-6	1.45 (1.07)	1.61	1.44	1.36
Me-8	1.50 (1.50)	1.01	1.57	1.04
Me-10	1.07 (1.19)	0.96	1.16	1.08
Me-12	1.00 (0.75)	0.82	1.13	1.06
Me-14	0.87 (0.96)	0.97	0.86	0.84
OH-3	*e* (1.08)	2.26		
OH-11	*e* (3.81)	2.85		
COMe			2.07	2.02
COMe			2.11	2.08

[a] Chemical shifts in ppm from internal TMS. [b] Solvent $CDCl_3$ at room temperature. Within the parentheses are reported the values in C_6D_6 at room temperature. [c] Solvent $CDCl_3$ at 250 K. [d] Solvent $CDCl_3$ at 263 K. [e] Not determined.

Compounds **13** and **14** show two free hydroxy groups which are linked at positions C(3) and C(11) of the macrolide ring. Accordingly, the acetyl derivatives **15** and **16** exhibit strong low-field chemical shift variations (0.5–1.5 ppm) of protons H(3) and H(11), while H(5) shows only small changes. These data confirm that in this case too, as previously reported by us[7] for (8*S*)-8-bromoerythronolide 5,9;6,9-spiroacetal B, the cyclization involves the hydroxyl groups OH(5) and OH(6) leading to the formation of 5- and 6-membered rings.

Table II. ^1H Coupling Constants (Hz) for Compounds 12–16

	12[a]	13[b]	14[c]	15[d]	16[e]
J(2,3)	10.4	10.5	0.5	11.2	11.0
J(3,4)	2.5	2.5	2.7	2.5	3.0
J(4,5)	0.8	0.5	3.5	0.5	2.0
J(7a,7b)	13.6	12.5	11.5	*f*	11.5
J(7a,F_8)		34.5		*f*	
J(7b,F_8)		14.0		*f*	
J(7a,8)			11.5		11.5
J(7b,8)			4.1		4.1
J(10,11)	6.0	6.2	0.5	3.5	1.5
J(11,12)	7.5	7.5	10.0	3.5	1.0
J(12,13)	4.0	3.8	2.0	2.5	3.5
J(13,14a)	11.4	11.0	9.0	7.5	9.5
J(13,14b)	2.6	2.5	5.0	7.5	5.5
J(14a,14b)	14.5	14.0	12.0	14.0	13.5
J(14,Me_{14})	7.4	7.5	7.2	7.5	7.5
J(2,Me_2)	6.7	6.9	7.3	6.5	6.6
J(4,Me_4)	7.6	7.5	8.0	6.5	7.2
J(8,Me_8)			7.0		6.1
J(F_8,Me_8)		21.2		21.5	
J(10,Me_{10})	7.6	7.5	7.4	7.3	7.0
J(12,Me_{12})	7.0	7.0	7.0	7.0	6.1
J(3,OH_3)	4.0	1.8	2.7		
J(11,OH_{11})	12.0	12.0	2.0		

[a] Taken from ref 7. [b] Solvent C_6D_6 at room temperature. [c] Solvent $CDCl_3$ at 250 K. [d] Solvent $CDCl_3$ at 263 K. [e] Solvent $CDCl_3$ at room temperature. [f] Not determined.

The *S* stereochemistry of C(8) and thus the pseudoequatorial orientation of the fluorine atom for the spiroacetal of the 8-fluoroerythronolide B **13** is also confirmed from the NMR data. In fact, the two methylene protons (H(7a) and H(7b) show coupling constants J(7a,F) and J(7b,F) of 34.5 and 14.0 Hz, respectively, indicating that, within the extremely rigid spiroacetalic structure, H(7a) is cis (dihedral angle near 0°) and H(7b) is trans (dihedral angle near 120°) to the fluorine atom.[8] Since H(7a) and H(5) are syn oriented as indicated by the strong NOE observed between them (12%), H(5) and F(8) must be in a trans pseudoaxial–pseudoequatorial relationship.

Conformational Studies. Most of the examined compounds exhibit conformational equilibria in solution. Two or more conformers in slow chemical exchange on the NMR time scale were clearly detected for **13–15** by the presence of broad resonances in the proton spectrum and by the observation of magnetization transfer in the NOE difference spectra. In contrast, the diacetyl derivative **16** is present essentially as one conformational isomer. We report here a conformational investigation on the most abundant conformer (75% or more) of each compound since the spectral analysis of the minor components is difficult due to the low intensity of their signals and to the extensive overlapping with the peaks of the main component. The values of the vicinal coupling constants of compounds **13–16**, together with those of the 8-bromoerythronolide B spiroacetal[7] **12** for comparison, are reported in Table II. The inspection of the table shows great variations of the coupling constants relative to the fragments C(2)–C(5) and C(10)–C(13) suggesting a marked conformational dependence of the macrolide ring on the substituents. A close similarity between solution conformation of Br and F derivatives may be inferred from them. On the contrary, replacing the halogen atom with a hydrogen results in a completely different conformation.

(8) Williamson, K. L.; Li Hsu, Y. F.; Hall, F. H.; Swager, S.; Coulter, M. S.; *J. Am. Chem. Soc.* **1968**, *90*, 6717.

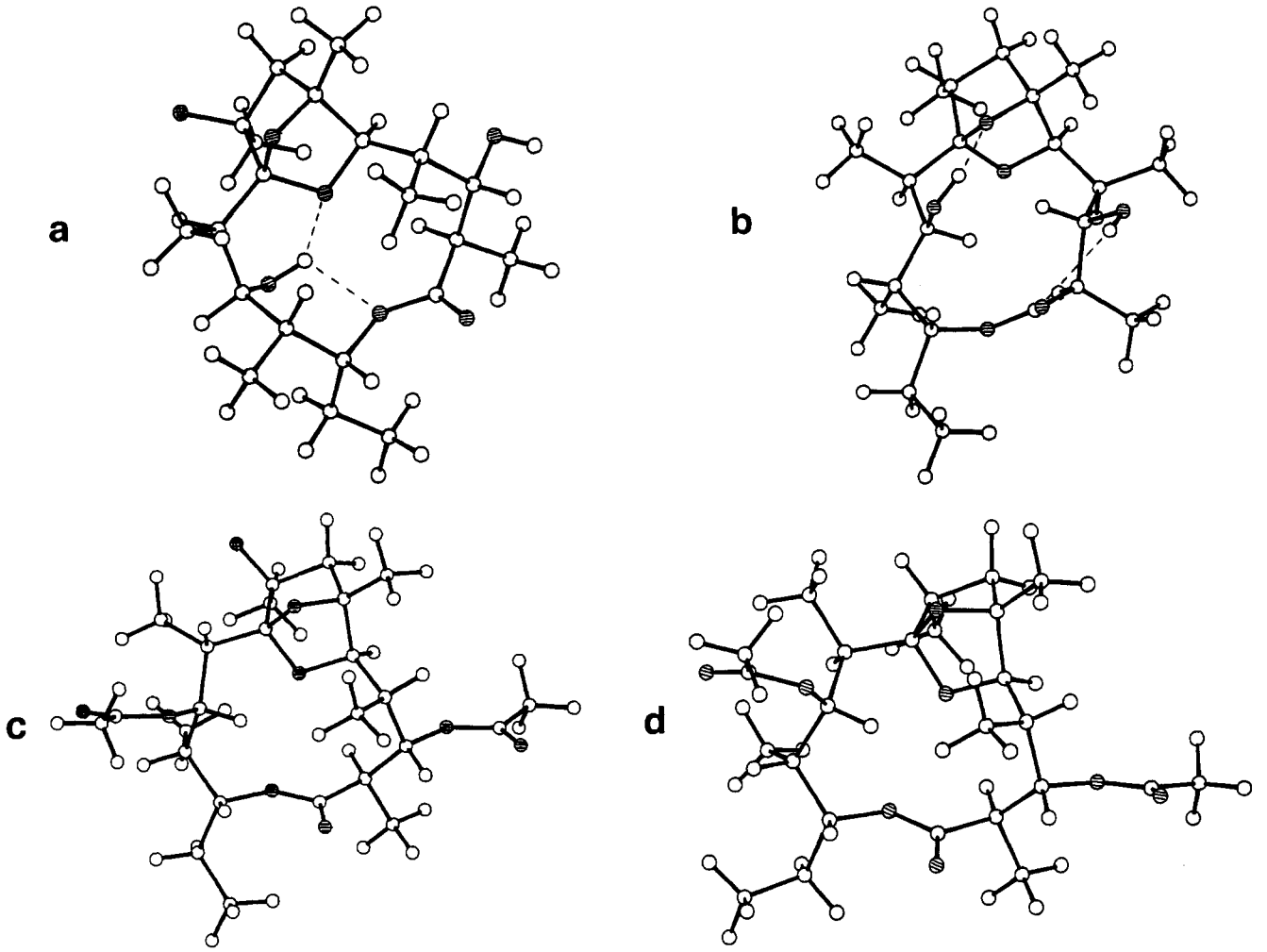


Figure 2. Stick and ball representation of the minimized structures consistent with the NOE data of (a) (8*S*)-8-fluoroerythronolide 5,9;6,9-spiroacetal B **13**, (b) erythronolide 5,9;6,9-spiroacetal B **14**, (c) 3,11-di(*O*-acetyl)-(8*S*)-8-fluoroerythronolide 5,9;6,9-spiroacetal B **15**, and (d) 3,11-di(*O*-acetyl)erythronolide 5,9;6,9-spiroacetal B **16**. The oxygen atoms are dashed and the fluorine atom is crosshatched.

Acetylation of the hydroxyl groups of **13** and **14** induces the ring to change to different conformational states.

To gain more insight on solution conformation of **13–16** a set of stationary NOE experiments were performed on the whole group of compounds. A selection of such NOEs involving protons lying on opposite sides of the 12-membered ring is reported in Table III. Moreover, to obtain a quantitative picture for the examined structures, we computed the H,H dihedral angles from vicinal coupling constants according to the generalized Karplus equation proposed by Altona et al.[9] and modeled all compounds using the MMX force field.[10] Since for each coupling constant there are four formal solutions of the Karplus equation, we selected those figures consistent with the NOE data. A first minimization was run using approximated distances from NOE data as constraints, then a second minimization was performed releasing all the constraints.

The calculated dihedral angles for the energy minimum conformation of 8-fluoroerythronolide B spiroacetal **13** are in very good agreement with the experimental ones obtained from the vicinal coupling constants (Table IV). This conformation, reported in Figure 2a, is practically the same one found for the 8-Br derivative **12** in the solid state:

Table III. Transannular Nuclear Overhauser Effects Observed for Compounds 12–16[a]

12[b]	{Me(4)}-H(12) (10%)
13	{Me(4)}-H(12) (10%)
14	{H(3)}-H(11) (17%); {H(11)}-H(2) (3%)
15	{Me(4)}-H(11) (3%); {H(2)-H(11) (1%)
16	{Me(4)}-H(11) (6%); {H(2)}-H(11) (1%)

[a] The irradiated nucleus is reported within brackets. [b] Taken from ref 7.

the hydroxyl proton OH(11) is hydrogen bonded to the oxygen atoms O(1) and O(5), as suggested also by its low-field chemical shift (3.81 ppm) and is oriented trans to H(11), as shown also by the large value (12 Hz) of the coupling constant $^3J_{OH(11),H(11)}$. The distance Me(4)–H(12) is 2.13 Å, well explaining the strong NOE observed between them. The agreement between calculated and experimental dihedral angles strongly suggests that the major conformer of (8*S*)-8-fluoroerythronolide 5,9;6,9-spiroacetal B is rigid.

On the contrary, the dihedral angles for the erythronolide spiroacetal B **14**, calculated from the energy minimum conformation which satisfies the NOE constraints, show several discrepancies with respect to the experimental ones (Table IV). A possible rationale is that the solution conformation corresponding to the most abundant species of **14** is the time average of two or more slightly different conformations in fast exchange on the NMR time scale. Regardless, the ball and stick model reported in Figure 2b

(9) Haasnoot, C. A. G.; De Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.

(10) PCMODEL Molecular Modeling Software, Serena Software, Box 3076, Bloomington, IN 47402-3076.

Table IV. Several Torsion Angles (deg) Relative to the Fragments C(2)–C(3)–C(4)–C(5) and C(10)–C(11)–C(12)–C(13) for Compounds 12–16 as Obtained from the Karplus Equation and from MMX Modeling (within Parentheses)

	12[a]	13	14	15	16
H(2)–H(3)	−174 (−177)	−176 (−174)	81 (84)	180 (−176)	180 (−176)
H(3)–H(4)	67 (67)	67 (67)	−110 (−127)	67 (71)	64 (72)
H(4)–H(5)	69 (77)	74 (75)	−58 (−58)	75 (74)	57 (75)
H(10)–H(11)	−44 (−44)	−44 (−44)	70 (75)	−60 (−80)	−75 (−74)
H(11)–H(12)	−140 (−141)	−140 (−140)	169 (164)	127 (133)	106 (130)
H(12)–H(13)	56 (57)	58 (58)	−55 (−54)	−51 (−71)	−44 (−76)

[a] Taken from ref 7.

should be a reasonable picture of the conformational preference of the macrolide ring of this compound. In fact, the modeled structure of **14** very well illustrates the outer orientation of Me(4) and the proximity of the H(3) and H(11) protons (calculated distance 2.2 Å). Two hydrogen bonds contribute to the stabilization of this conformation, i.e., OH(11)---O(6) and OH(3)---CO with a proton–oxygen distance of 1.9 and 2.3 Å, respectively. This is consistent with the chemical shift variations of these hydroxyl protons showing a very small dependence on concentration itself.

The two diacetyl derivatives **15** and **16** show rather great discrepancies between the dihedral angles calculated from the modeled structures and those obtained from the vicinal coupling constants (Table IV). This behavior strongly suggests that the ring conformation of **15** and **16** is not rigid and some fast motion should occur along the C(2)–C(5) and C(10)–C(13) fragments. Although the experimental dihedral angles are not completely simulated, the distances between H(11) and Me(4) and between H(11) and H(2) (ca. 3 and 3.9 Å, respectively) are consistent with the observed NOEs. In summary, the main conformational features of the acetyl derivatives are the outer position of the acetyl substituents and the H11, H(2) and Me(4) groups pointing toward the inner part of the molecule (Figure 2c,d).

The results discussed above for compounds bearing free hydroxyl groups show that the substituent placed in position 8, thus on a conformationally rigid fragment of the molecule, can drive the 12-membered macrolide ring toward each conformer. In order to find a rationale for the observed conformational flexibility it is useful to compare directly the erythronolide 5,9;6,9-spiroacetal B **14** and the bromo derivative **12**. The most abundant conformer of **14** displays the H atom in position 8 and the methyl group in position 10 in an eclipsed relationship. In fact, the dihedral angle H(8)–C(8)–C(9)–C(10) is found to be 45.8° and the dihedral Me(10)–C(10)–C(9)–C(8) −44.7°. Although the distance between H(8) and the carbon atom of Me(10) is slightly less than the sum of their van der Waals radii, this arrangement should be energetically favorable since it allows the formation of the two hydrogen bonds OH(11)---O(6) and OH(3)---CO. In the bromo derivative the different chain arrangement causes a partial release of the steric strain between Me(10) and the bromine atom directly attached to C(8), which actually gain a staggered conformation. This is clearly shown by the values of calculated dihedral angles: Br–C(8)–C(9)–C(10) 45.8° and Me(10)-C(10)-C(9)-C(8) −112.9°. Moreover, the distance between Br and Me(10) is 3.81 Å corresponding to the sum of their van der Waals radii.

Hydrogen bonds and sterical hindrance can reasonably be considered as the reasons for the conformational behavior of **12** and **14**. Nevertheless, it is not immediately clear why the most abundant conformer of 8-fluoroerythronolide 5,9;6,9-spiroacetal B **13** is actually identical to that of the bromo derivative **12** and completely different from that of the erythronolide B spiroacetal **14**. Fluorine atom is commonly believed to be nearly isosteric with hydrogen,[11] thus suggesting that **13** and **14** should have the same conformational behavior. An alternative hypothesis is that the highly electronegative fluorine atom polarizes in a different way, via a through space field effect,[12] the two C–O bonds of the spiroacetal group causing a variation of the charge density at the two oxygen atoms. A hydrogen bond arrangement as present in the bromo derivative **12** might be in this case favored with respect to the one exhibited by the erythronolide B spiroacetal **14** itself.

For the acetylated compounds **15** and **16** the two acetyl groups move toward outer positions since no hydrogen bonds can be established in this case. Consequently, Me(4) is pseudoaxially oriented and Me(10) and the substituent at C(8) exhibit an intermediate relationship between that of compound **12** (staggered) and **14** (eclipsed).

Experimental Section

Compounds **13–16** were synthesized following the procedure described in refs 5 and 6. **13** is a mixture of three conformers in slow exchange on the NMR time scale in a ratio of ca. 76:14:10; **14** and **15** are composed of two conformational isomers in a ratio of ca. 80:20; **16** gives rise in solution to one conformer only. The proton spectra have been acquired on the Bruker CXP 300 or AC 250 instruments. The 2D $^1H,^1H$ COSY spectra were run with a sweep width of 1750 Hz using 2048 data points in f_2, a 90° pulse of 9 s and a relaxation delay of 2 s. The long-range $^1H,^1H$ COSY experiments with a delay period of 0.07 s were also performed to emphasize the correlations due to the small couplings. The proton nuclear Overhauser effects were obtained from 2D NOESY experiments or from monodimensional NOE difference spectra. Oxygen was partially removed from the NMR tube by bubbling nitrogen through the solution for 10–15 min. The 2D phase-sensitive NOESY experiments were acquired with a mixing time of 0.7–1.0 s and 2K data points. Typically, for the ^1H NOE difference spectra, a set of five to seven experiments were performed with the decoupler on-resonance and then subtracted from a control spectrum with the decoupler off-resonance. The irradiation time was 2–3 s and the relaxation delay 5–7 s. Some experiments have been carried out at low temperature (250–270 K) to minimize the magnetization transfer due to the chemical exchange between the major and the minor conformational isomers. The OH-3 hydroxyl group of **19** in benzene falls in a crowded region of the spectrum and was evidenced using a magnetization transfer experiment performed irradiating the water signal at ca. 0.5 ppm and then subtracting the spectrum from a reference one with the decoupler off-resonance.

Dihedral angles were computed on a personal computer from the vicinal coupling constants according to the Karplus equation developed by Altona et al.[9] Energy minimizations were performed on a personal computer (Compaq 386) using the PCMODEL molecular modeling software[10] and a dielectric constant of 1.5.

Registry No. **13**, 137743-00-1; **14**, 137743-01-2; **15**, 137743-02-3; **16**, 137768-18-4.

(11) Goldman, P. *Science* **1969**, *164*, 1123.
(12) Topsom, R. D. In *Progress in Physical Organic Chemistry*; Vol. 12, Taft, R. W., Ed.; Interscience: New York, 1976, Vol. 12, pp 2–6.