

A Novel Aspect of the Reaction of Ivermectin Aglycon Producing 13-Substituted-14*Z* Derivative

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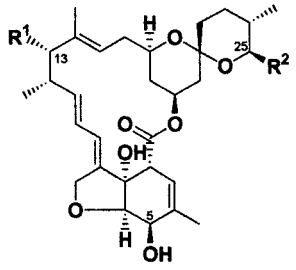
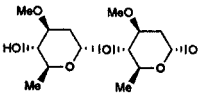
During research to synthesize 13-deoxy-13β-substituted ivermectin aglycons, unexpected unique products, 13β-substituted-14*Z* ivermectin aglycons, were obtained. We will report the results of that series of studies and a possible reaction mechanism.

Milbemycins, which are isolated from *Streptomyces hygroscopicus*,[1] have attracted a great deal of interest, as they are known to be potent anthelmintic products.[2] Ivermectin (22,23-dihydroavermectin)[3] is also known to possess activity against endoparasites similar to that of milbemycins. These two compounds have a similar structure, both having a 16-membered ring. The significant structural differences are the substituents at the 13-position and the 25-position. That is, ivermectin has a 4-*O*-α-L-oleandrosyl-α-L-oleandrosyl group at the 13-position with an α-configuration, while milbemycin has no functional group in this position (Fig. 1), and ivermectin has an *s*-butyl group at the 25-position while milbemycins have a methyl or ethyl group there.

It was reported by Mrozik et al.[4] that the substituent at the 13-position greatly contributes to the anthelmintic activity of ivermectins. Thus a great number of 13-substituted milbemy-



	R^1	R^2
Milbemycin A3	H	Me
Milbemycin A4	H	Et
Ivermectin		mixture of s-Bu and i-Pr

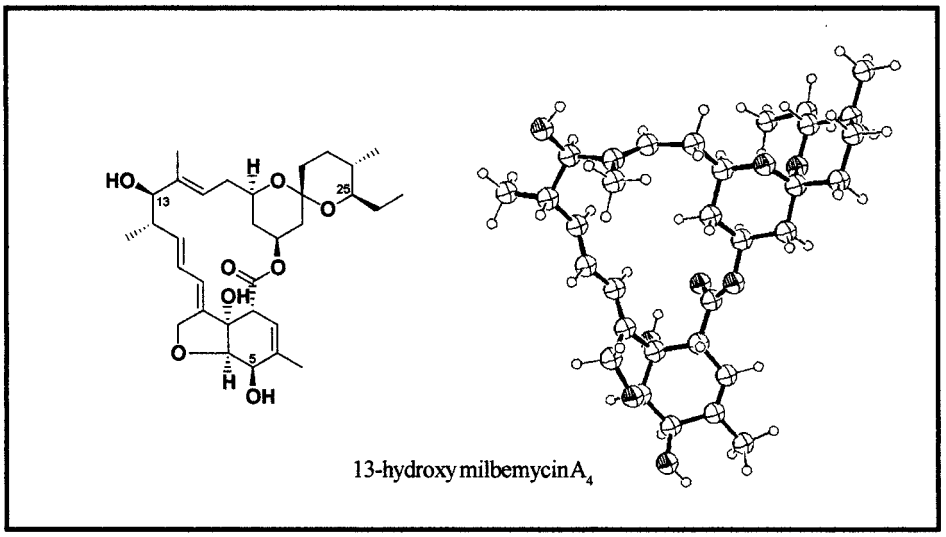


Fig. 1. Structures of milbemycins and ivermectin. The 3D structure of 13-hydroxy milbemycin A_4 was calculated by MM2 and MOPAC based on the X-ray structure of milbemycin A.

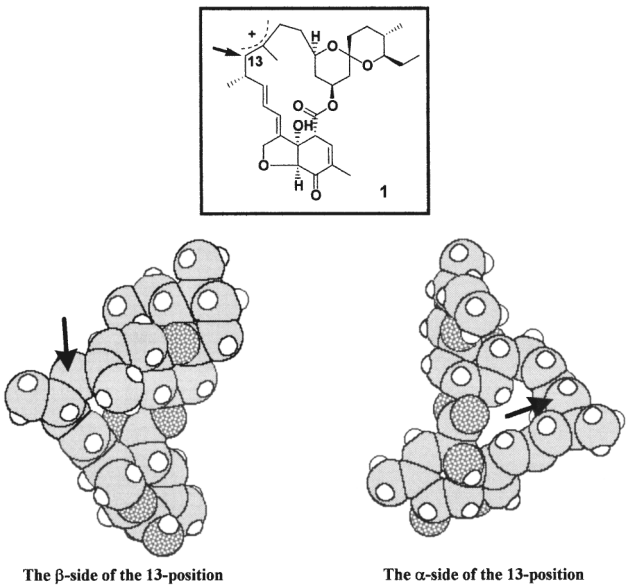


Fig. 2. Space filling model of the usual allyl cation.

cin derivatives also have been synthesized and their activities evaluated, and they were found to have anthelmintic activity as potent as ivermectin, although there is a difference between their configurations at the 13-position, α (ivermectin) and β (13-substituted milbemycins).

The structure-activity relationships of milbemycin and ivermectin derivatives have also been studied by examining the substituent at the 25-position.[5] That is, the derivatives of milbemycin A_3 (C-25 methyl), milbemycin A_4 (C-25 ethyl), and ivermectin (C-25 *s*-butyl) were synthesized and their activities were compared.[5] In the course of this study, it was necessary for us to establish a method to synthesize 13β-substituted-13-deoxy-ivermectin aglycon from ivermectin aglycon, because it is known that the 13α-substituted derivative has less activity than the 13β-substituted derivative.[4] There seemed to be no difficulty in this synthesis according to these reasons: 1) We have established a practical method to synthesize 13β-substituted milbemycin derivatives from 15-hydroxymilbemycin via allyl cation **1** (Fig. 2) in our previous paper;[6] 2) The reaction of the 13α-hydroxyivermectin derivative was also likely to proceed in a similar manner via allyl cation to produce the desired 13β-substituted derivative; and 3) The steric hindrance of the α-side of the reaction site (Fig. 2) would also facilitate obtaining the desired 13β-substituted derivatives from the 13α-hydroxy derivative.

Regardless of the predictions above, the reaction did not proceed as we expected. It gave a mixture of three derivatives (Fig. 3): a small quantity of desired 13β-14*E* derivative **2**, some undesired 13α-14*E* derivative **3**, and, to our surprise, 13β-14*Z* derivative **4**. Those structures were determined by NMR spectra and mass spectra:[7] **2** has a distinct doublet as α-C-13H at 4.91 ppm, with a large coupling constant (10.2 Hz);[8] **3** has a distinct multiplet as β-C-13H at 5.05 ppm;[8] and **4** has a doublet as α-C-13H at 5.23 ppm, approximately 0.3 ppm lower than 14*E*-derivative,[9] and also has a multiplet as C-17H at 3.82 ppm, approximately 0.3 ppm lower than 14*E*-derivative.[9] Thus we examined the series of reactions to obtain the desired product as described below.

Results

Reaction at Room Temperature. First of all, a reaction of ivermectin aglycon (**5**) and carboxylic acid **6** at room temperature was examined. When the reaction took place at room temperature, the products were a mixture of three derivatives: **2**, **3**, and **4** (Fig. 3). The ratio of these three derivatives was **2**:**3**:**4** = 8:14:17 according to the analysis of the peak areas by HPLC, showing that the desired product was obtained at very low yield.

Time Course Study of the Reaction. Then, to maximize the ratio of the desired product, time course analysis of the ratio of each compound was carried out. At the time points of 0, 5, 10, 30, 60, 120, and 180 min, a part of the reaction mixture was extracted and the ratio of the three compounds (**2**, **3**, and

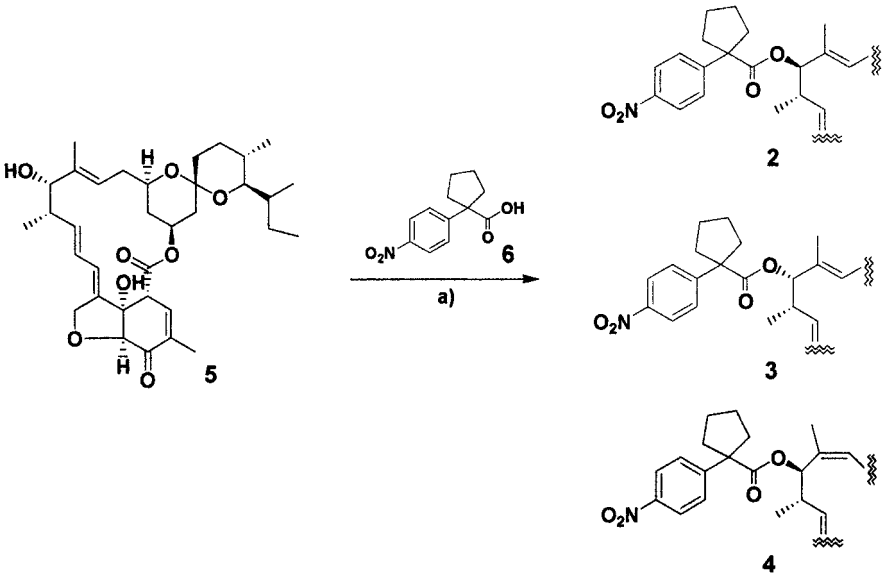


Fig. 3. Reaction of ivermectin aglycon. a) CF_3SO_3H/CH_2Cl_2.

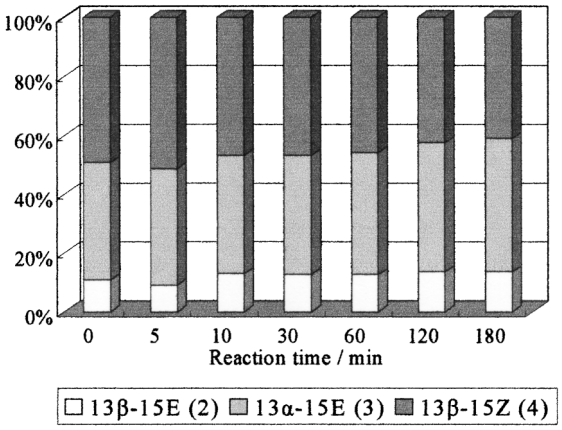


Fig. 4. Relative ratios of 2, 3, and 4 under room temperature.

4) was analyzed by HPLC (Fig. 4). Unsatisfactorily, the desired product **2** was again obtained in low yield. Here, the ratio of 13β-14*Z* derivative **4** was inversely dependent on the reaction time, thus transformation of 13β-14*Z* derivative **4** to 13α-14*E* derivative **3** was presumed.

Effect of the Reaction Temperature. If a transformation from **4** to **3** occurs, the reaction temperature could have an effect on the ratio of **2**, **3**, and **4** because the transformation should take place via cation(s). Also the ratio of the desired product **2** was expected to rise when the reaction took place at higher temperature because its structure is presumed to be thermodynamically stable. Thus, the ivermectin aglycon (**5**) and carboxylic acid **6** were mixed under the same reaction conditions as above, except the temperature was 40 °C, and time course analysis of the ratio of the three compounds was examined again. This time, at the time points of 5, 10, 20, 60, 120, and 180 min, a part of the reaction mixture was extracted and the ratio of the three compounds was analyzed by HPLC (Fig. 5). Compared to the result at room temperature, the ratio of 13β-14*Z* derivative **4** was apparently decreased, and the ratio of 13α-14*E* derivative **3** was increased. Although the yield of the desired compound was not satisfactory, the transformation of **4** to **3** was enhanced here.

Conversion of the 14*Z* Derivative to the 14*E* Derivative. As transformation from 13β-14*Z* derivative **4** to 13α-14*E* derivative **3** was suggested, 13β-14*Z* derivative **4** itself was purified and examined to see whether the transformation would take place (Fig. 6). As we expected, both **2** and **3** were obtained almost instantly when **4** was subject to the reaction conditions (Fig. 7). Thus, this result strongly supports that there must be conversion of **4** into **3**.

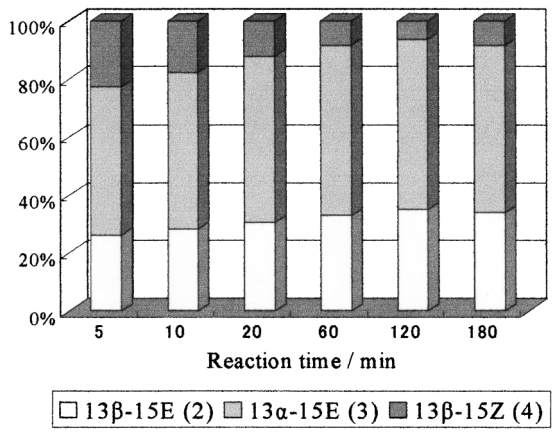


Fig. 5. Relative ratios of 2, 3, and 4 at 40 °C.

Conversion of the 13α-14*E* Derivative to Other Derivatives. Regarding the results in the reactions above, also the transformation from 13α-14*E* derivative **3** to other products (**2** and **4**) was suggested. So 13α-14*E* derivative **3** itself was isolated and examined to see whether it produces those compounds under the same reaction conditions as mentioned above (Fig. 6). The result showed that transformation occurred from the 13α-14*E* derivative **3** to products **2** and **4**, although the ratio of the transformation was lower than that from 13β-14*Z* derivative **4** (Fig. 8).

Conversion of the 14*Z* Derivative in the Presence of Carboxylic Acid 7. As those conversions were presumed to be via cation(s), the reaction of 13β-14*Z* derivative **4** with carboxylic acid **7** was examined to verify that it was due to intermolecular reaction, not intramolecular reaction (Fig. 6). As the α-position of the carbonyl group in **7** is less hindered than that in **6**, it was predicted that exchange of the acyloxy group at the 13-position would take place readily. To our satisfaction, exchange certainly took place to produce **8** and **9** as the only products; **2**, **3**, **4**, and **10** were not detected (Fig. 6).

Reaction of 13α-Hydroxy Derivative with Carboxylic Acid 7. To compare the result of the reaction of **4** with carboxylic acid **7**, 13α-hydroxy derivative **5** was used as a substrate in the reaction conditions with carboxylic acid **7**. The products were in the ratio of 55% 13α-14*E* derivative **9**, 30% 13β-14*Z* derivative **8**, and 10% 13β-14*E* derivative **10** according to the analysis of the peak areas by HPLC.

Discussion

According to the results above, we gave up obtaining the 13β-substituted-13-deoxy ivermectin aglycon from ivermectin aglycon at high yield. But in that series of the studies, we encountered a very interesting compound, the 13β-substituted-14*Z* derivative. Thus we suggest some hypotheses about the reaction mechanism to obtain the 14*Z* derivative **4** as follows.

The Possibility of the Conversion between *E*-Derivative and *Z*-Derivative. It is very likely that there is a path, which enables the conversion from *E* to *Z*, and the path is completely different from the one that is in the reaction of 13β-hydroxy milbemycin. If the reaction occurs in the same path via the allyl cation as reported in our previous paper,[6] the product should be only 13β-substituted product regarding its steric hindrance.

Possibility of a Homoallylic Cation. Similar rearrangement at the 13-position was also examined by Merck researchers using the 13α-substituted derivative as a substrate.[4] In their report, the configuration at the 13-position was retained when solvolysis took place on the 13α-substituted derivative, and a homoallylic cation was suggested as an explanation of the retention of the stereochemistry. But they did not mention any unusual by-products such as the 13β-substituted-14*Z* derivative. Besides, the mechanism for obtaining these by-products cannot be explained by a homoallylic cation.

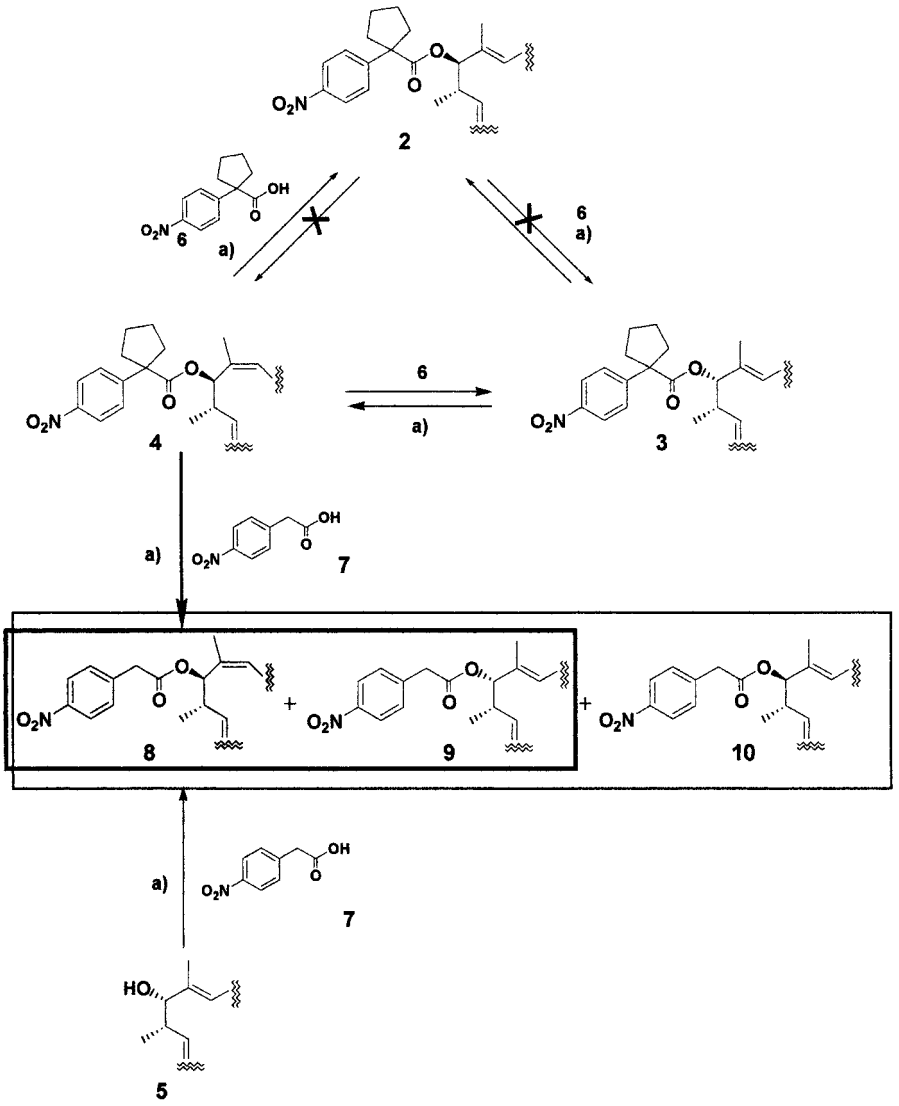


Fig. 6. Examined reactions between the products. a) CF_3SO_3H/CH_2Cl_2.

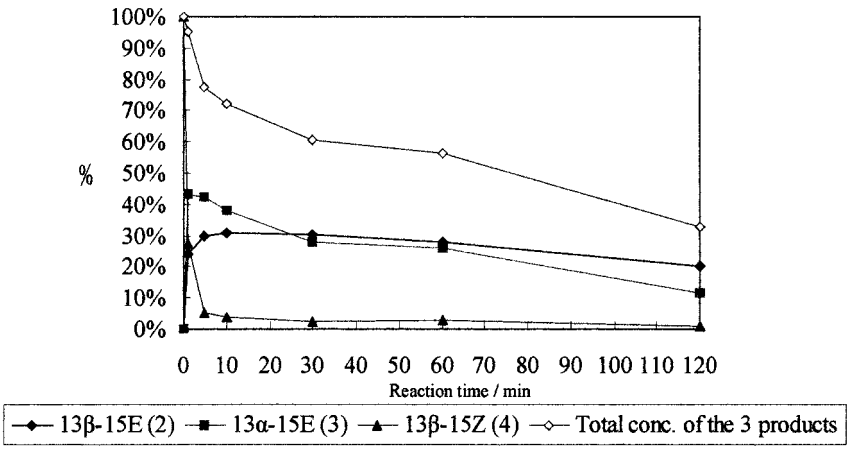


Fig. 7. Actual concentrations of 2, 3, and 4 and their total concentration in the reaction mixture. The 13β-substituted-15*Z* (**4**) derivative was used as a substrate. The reaction took place under room temperature.

Mechanism to Produce the Unique Configurations. Although it seems very difficult to change the configuration from *E* to *Z* as presumed above, we regard that it is the inflexibility of the rigid 16-membered ring (Fig. 1) that causes a different

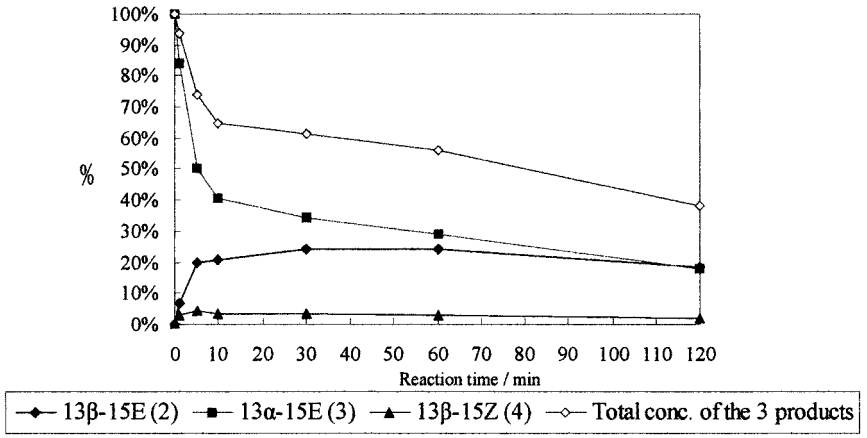


Fig. 8. Actual concentrations of 2, 3, and 4 and their total concentration in the reaction mixture. The 13α-substituted-15*E* (**3**) derivative was used as a substrate. The reaction took place under room temperature.

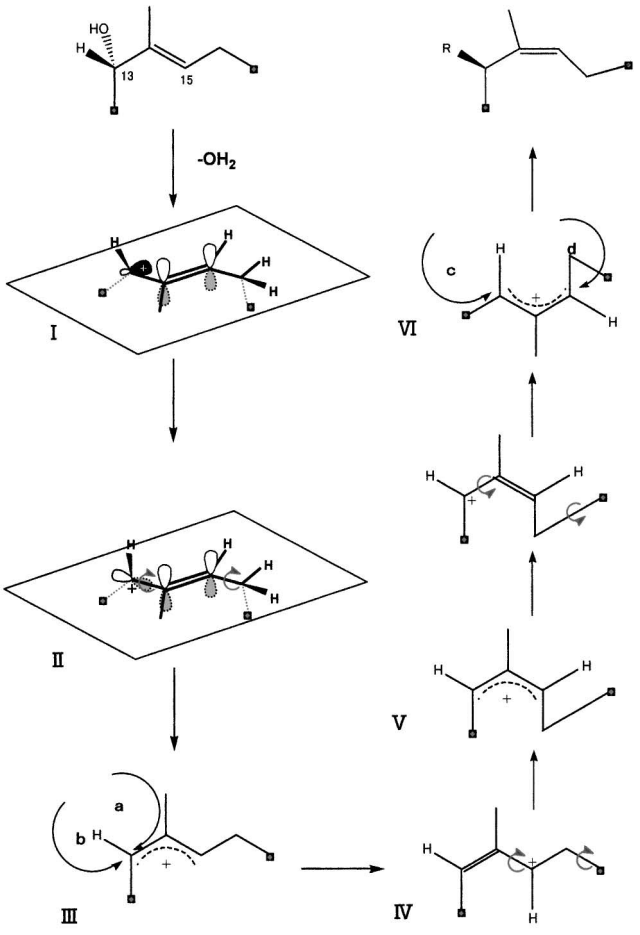


Fig. 9. Presumed reaction mechanism producing 15-*Z* derivative. ◘ This mark shows the carbon, which is almost impossible to move because of the rigid 16-membered ring. The allyl cation III is different from the allyl cation, which is shown in Fig. 2.

reaction from the one in the linear molecule. The hydroxy group at the 13-position was protonated and eliminated to produce the cations. If the hydroxy group is in the β-position, the vacant orbital would be parallel to the p orbital of the adjacent double bond, thus it would produce the allyl cation readily, which configuration is almost the same configuration as the 13β-hydroxy derivative to produce the corresponding product **2**. On the other hand, if the hydroxy group is in the α-position, the situation is completely different from the one in the β-position (Fig. 9). That is, the conversion of the configuration from *E* to *Z* occurs supposedly in the following manner: First, the hydroxy group at the 13α-position is protonated and eliminated (Fig. 9-I). The vacant orbital is almost vertical to the p orbital of the adjacent double bond, thus it is unable to produce the allyl cation readily, so the C-13 changes its orbital from sp^3 to sp^2 (Fig. 9-II). Then, as the p orbital at C-13 is vertical to the adjacent p orbital, a bond pair between C-13 and -14, and C-15 and -16, rotates to bring about the conjugation between the cation and the double bond (Fig. 9-II) to produce the allyl cation (Fig. 9-III). Those two bonds are required for simultaneous rotation because the 16-membered ring is too rigid to allow rotation of the bonds. At this point, the α-side of C-13 is less hindered than the β-side; thus the 13α-substituted product should be produced here (path a), and maybe a small amount of 13β-product is produced despite the steric hindrance (path b). Second, the double bond migrates to the bond between C-13 and C-14 (Fig. 9-IV), then a bond pair between C-14 and -15 and C-16 and -17 rotates to make a *Z*-like configuration (Fig. 9-IV to V). However, this configuration (V) is also very unstable. Thus the bond pair, between C-13 and -14 and C-16 and -17 rotates again to counter the strain in the 16-membered ring, resulting in production of the allyl cation (Fig. 9-VI). At this point, the β-side of C-13 is less hindered than the α-side, allowing only the 13β-14*Z*-product (path c). The 15-substituted product could be obtained (path d) here, but it would readily convert into the 13-subsituted product as we reported in our previous paper.[6]

Discussion on the Cations. According to the result of the reaction between **4** and **7** (Fig. 6), the cation(s) are certainly produced during the reaction, although the cation(s) themselves cannot be isolated or identified.

Summary. In the course of our study, we encountered a unique reaction producing a 14*Z* derivative that is usually not observed. Although the exact configurations of the cations were not verified, a plausible reaction mechanism to obtain the

14*Z*-ivermectin derivatives was suggested.

Experimental

The Reaction of 5 with 6. Carboxylic acid **6** (160.9 mg, 0.68 mmol) was dissolved in dichloromethane (3.0 mL), trifluoromethanesulfonic acid (10 µL) was added, and the mixture was stirred for 5 min at room temperature. Then a solution of **5** (100 mg, 0.17 mmol) in dichloromethane (2 mL) was slowly added and the mixture was stirred at room temperature for 40 min. The reaction mixture was diluted with a mixture of ethyl acetate and 4% aqueous solution of $NaHCO_3$ to quench the reaction, extracted with dichloromethane, washed with a 10% aqueous solution of $NaHCO_3$ and with water, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on ODS with eluent (100% acetonitrile) to obtain a mixture of **2**, **3**, and **4**. The ratio of **2**, **3**, and **4** was estimated at **2**:**3**:**4** = 8:17:14 by analyzing the area ratio of the HPLC spectrum. The mixture was chromatographed on ODS with eluent (100% acetonitrile), again to isolate the pure part of each of the compounds. The purified compounds **2**, **3**, and **4** were analyzed by NMR to identify their structure. **2**; ^{1}H NMR δ 0.77 (3H, d, J = 5.9 Hz, C-24 CH_3), 0.84 (3H, d, J = 6.6 Hz, C-12 CH_3), 0.94 (3H, t, J = 7.7 Hz, C-28 H), 1.88 (3H, s, C-4 CH_3), 3.14 (1H, m, C-25 H), 3.53 (1H, m, C-2 H), 3.57 (1H, m, C-17 H), 3.83 (1H, s, C-6 H), 3.96 (1H, broad s, C-7 OH), 4.69 and 4.73 (2H, ABq, J = 14.4 Hz, C-8 CH_2), 4.91 (1H, d, J = 10.2 Hz, C-13 H), 5.77 (1H, dd, J = 14.3 and 11.7 Hz, C-10 H), 5.85 (1H, dt, J = 11.7 and 2.4 Hz, C-9 H), 6.55 (1H, s, C-3 H), 7.50 (2H, d, J = 8.8 Hz, Ph H), 8.16 (2H, d, J = 8.8 Hz, Ph H). **3**; ^{1}H NMR δ 0.88 (3H, d, J = 6.6 Hz, C-12 CH_3), 1.01 (3H, t, J = 7.3 Hz, C-28 H), 1.89 (3H, s, C-4 CH_3), 3.16 (1H, m, C-25 H), 3.84 (1H, s, C-6 H), 3.94 (1H, broad s, C-7 OH), 4.69 and 4.74 (2H, ABq, J = 14.5 Hz, C-8 CH_2), 4.78 (1H, m, C-15 H), 5.05 (1H, m, C-13 H), 5.39 (1H, m, C-19 H), 5.47 (1H, dd, J = 14.7 and 10.2 Hz, C-11 H), 5.71 (1H, dd, J = 14.7 and 11.0 Hz, C-10 H), 5.88 (1H, dt, J = 11.0 and 2.2 Hz, C-9 H), 6.58 (1H, s, C-3 H), 7.59 (2H, d, J = 8.8 Hz, Ph H), 8.19 (2H, d, J = 8.8 Hz, Ph H). **4**; ^{1}H NMR δ 0.82 (3H, d, J = 6.5 Hz, C-12 CH_3), 0.90 (3H, t, J = 7.3 Hz, C-28 H), 1.88 (3H, s, C-4 CH_3), 3.11 (1H, m, C-25 H), 3.53 (1H, m, C-2 H), 3.82 (1H, m, C-17 H), 3.84 (1H, s, C-6 H), 4.63 (1H, broad s, C-7 OH), 4.68 and 4.74 (2H, ABq, J = 14.6 Hz, C-8 CH_2), 5.23 (1H, d, J = 11.0 Hz, C-13 H), 5.25 (1H, dd, J = 13.6 and 10.6 Hz, C-11 H), 5.35 (1H, m, C-19 H), 5.41 (1H, m, C-15 H), 6.50 (1H, s, C-3 H), 7.50 (2H, d, J = 8.8 Hz, Ph H), 8.17 (2H, d, J = 8.8 Hz, Ph H).

The Reaction of 4 to 3. Carboxylic acid **6** (29.3 mg, 0.13 mmol) was dissolved in dichloromethane (3.0 mL), trifluoromethanesulfonic acid (5 µL) was added, and the mixture was stirred for 5 min at room temperature. Then a solution of **4** (25.0 mg, 0.03 mmol) in dichloromethane (1 mL) was slowly added and the mixture was stirred at 40 °C for 120 min. A part of the reaction mixture was extracted, diluted with a mixture of ethyl acetate and 4% aqueous solution of $NaHCO_3$, and analyzed by HPLC to identify the product **3**.

The Reaction of 3 to 2 and 4. Carboxylic acid **6** (23.5 mg, 0.10 mmol) was dissolved in dichloromethane (3.0 mL), trifluoromethanesulfonic acid (5 µL) was added, and the mixture was stirred for 5 min at room temperature. Then a solution of **3** (20.0 mg, 0.025 mmol) in dichloromethane (1 mL) was slowly added and the mixture was stirred at 40 °C for 120 min. A part of the reaction mixture was extracted, diluted with the mixture of ethyl acetate and 4% aqueous solution of $NaHCO_3$, and analyzed by HPLC to identify the products **2** and **4**.

The Reaction of 4 with 7. Carboxylic acid **7** (451.8 mg, 2.49 mmol) was dissolved in dichloromethane (10 mL), trifluoromethanesulfonic acid (30 µL) was added, and the mixture was stirred for 5 min at room temperature. Then a solution of **4** (500 mg, 0.62 mmol) in dichloromethane (3 mL) was slowly added and the mixture was stirred at room temperature for 40 min. The reaction mixture was diluted with a mixture of ethyl acetate and 4% aqueous solution of $NaHCO_3$ to quench the reaction, extracted with ethyl acetate, washed with a 10% aqueous solution of $NaHCO_3$, and with water, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on ODS with eluent (90% acetonitrile) to obtain a mixture of **8** (122.6 mg, 25.8% yield) and **9** (128.4 mg, 27.4% yield). The compounds **8** and **9** were analyzed by NMR to identify their structure. **8**; ^{1}H NMR δ 0.82 (3H, d, J = 6.7 Hz, C-12 CH_3), 0.90 (3H, t, J = 7.4 Hz, C-28 H), 1.89 (3H, s, C-4 CH_3), 3.10 (1H, m, C-25 H), 3.55 (1H, m, C-2 H), 3.74 (2H, s, benzyl H), 3.83 (1H, m, C-17 H), 3.86 (1H, s, C-6 H), 4.65 (1H, broad s, C-7 OH), 4.71 and 4.77 (2H, ABq, J = 14.7 Hz, C-8 CH_2), 5.36 (1H, d, J = 10.9 Hz, C-13 H), 5.47 (1H, m, C-5 H), 6.51 (1H, s, C-3 H), 7.45 (2H, d, J = 8.7 Hz, Ph H), 8.20 (2H, d, J = 8.7 Hz, Ph H). **9**; ^{1}H NMR δ 0.88 (3H, d, J = 6.6 Hz, C-12 CH_3), 0.98 (3H, t, J = 7.3 Hz, C-28 H), 1.90 (3H, s, C-4 CH_3), 3.19 (1H, m, C-25 H), 3.57 (1H, m, C-2 H), 3.63 (1H, m, C-17 H), 3.86 (1H, s, C-6 H), 3.87 and 3.83 (2H, ABq, J = 15.4 Hz, benzyl H), 4.72 and 4.76 (2H, ABq, J = 14.5 Hz, C-8 CH_2), 4.84 (1H, m, C-15 H), 5.17 (1H, m, C-13 H), 5.36–5.44 (1H, m, C-19 H), 5.64 (1H, dd, J = 14.8 and 10.0 Hz, C-11 H), 5.78 (1H, dd, J = 14.8 and 11.1 Hz, C-10 H), 5.91 (1H, dt, J = 11.1 and 2.3 Hz, C-9 H), 6.58 (1H, m, C-3 H), 7.51 (2H, d, J = 8.7 Hz, Ph H), 8.23 (2H, d, J = 8.7 Hz, Ph H).

The Reaction of 5 with 7. Carboxylic acid **7** (144.9 mg, 0.8 mmol) was dissolved in dichloromethane (3.0 mL), trifluoromethanesulfonic acid (10 µL) was added, and the mixture was stirred for 5 min at room temperature. Then a solution of **5** (116.9 mg, 0.2 mmol) in dichloromethane (2 mL) was slowly added and the mixture was stirred at room temperature for 60 min. A part of the reaction mixture was extracted, diluted with a mixture of ethyl acetate and 4% aqueous solution of $NaHCO_3$, and analyzed by HPLC to identify the products **8**, **9**, and **10**. The structure of **10** was compared to the authentic sample to identify its structure. **10**; ^{1}H NMR δ 1.89 (3H, s, C-4 CH_3), 3.14 (1H, m, C-25 H), 3.55 (1H, m, C-2 H), 3.60 (1H, m, C-17 H), 4.72 and 4.76 (2H, ABq, J = 14.3 Hz, C-8 CH_2), 4.98 (1H, d, J = 10.9 Hz, C-13 H), 6.57 (1H, m, C-3 H).

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7 The 14*Z* configuration has already been proved to be produced during the reaction of 15-hydroxymilbemycin by O'Sullivan,[9] although the 13-substituted-14*Z* derivative was not obtained in their reaction.

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