# SOME BASE-CATALYZED REACTIONS OF *NOR*-CLERODANE DERIVATIVES AND THEIR ANTIFEEDANT ACTIVITY

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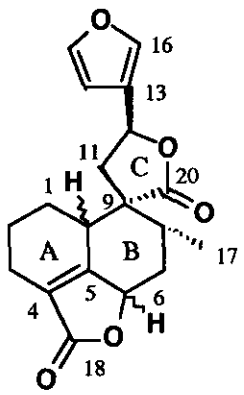
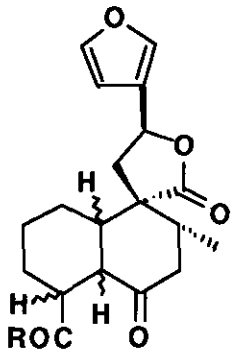
Abstract — By base-catalyzed reaction of a *nor*-clerodane diterpene, teucvidin (**1**), several *cis*- (**5**, **6**) and *trans*-clerodane derivatives (**3**, **7**, **8**) were obtained. Their structures including stereochemistry were established by spectroscopic means and X-ray analysis of **5**, and by correlation to the known products. The formation of these compounds implied that a different kind of basic reagents had influence on the stereochemistry of reactive products. Among the compounds obtained, **7** showed the most potent antifeedant activity to larvae of *Leucania separata*.

The genus of *Teucrium* (Labiatae) is so far the most abundant natural source of *neo*-clerodane and 19-*nor*-*neo*-clerodane diterpenoids.[1] In last few years these compounds have drawn much attention due to the various biological activities such as insect antifeedant, antifungal, antitumor, antimicrobial and molluscicidal.[2-6] In continuation of our studies of *nor*-clerodane diterpenoid chemistry, we were interested in the correlation of the structure and the antifeedant activity. In this paper we report some base-catalyzed transformations of a *nor*-clerodane diterpene, teucvidin (**1**) isolated from *Teucrium quadrifarium*,[7] and the results of antifeedant activity assessed against larvae of *Leucania separata* Walker.

## RESULTS AND DISCUSSION

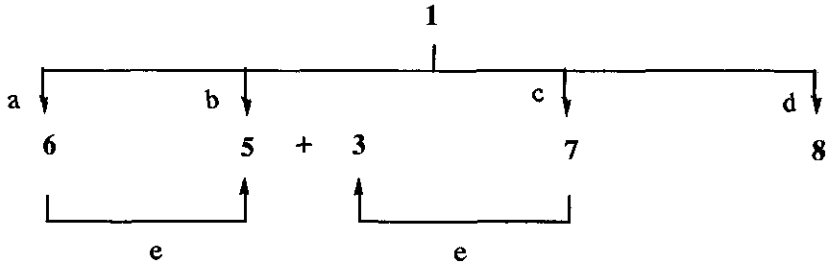
Ester exchange reaction of teucvidin (**1**)[8] and its isomer teucvin (**2**)[9] with $Na_2CO_3$ in MeOH resulted in formation of keto esters (**3**)[8,10] and (**4**)[9] possessing a *trans*-clerodane skeleton. Through our further investigation of the reaction, **1** was found to yield another new minor substance (**5**) accompanied by **3** in a ratio of *ca.* 1 : 10.




**1** 6α-H, 10α-H (teucvidin)
**2** 6β-H, 10β-H (teucvin)

**3** R = OMe, 4α-H, 5β-H, 10α-H
**4** R = OMe, 4β-H, 5α-H, 10β-H
**5** R = OMe, 4α-H, 5α-H, 10α-H
**6** R = OH, 4α-H, 5α-H, 10α-H
**7** R = OH , 4α-H, 5β-H, 10α-H
**8** R = $NH_2$, 4α-H, 5β-H, 10α-H




a: *t*-BuOK/*t*-BuOH; reflux, b: $Na_2CO_3$/MeOH; reflux, c: 0.5N NaOH/50% MeOH; 70°C, d: $NH_4OH$/MeOH, 50°C, e: $CH_2N_2/Et_2O$

Compound (**5**) had a molecular formula $C_{20}H_{24}O_6$ and its ir spectrum was similar to that of **3**, suggesting that **5** is a stereoisomer of **3**. Comparison of the $^1H$ and $^{13}C$ nmr spectral data with those of **3** revealed that the chemical shift and *J* value based on a proton at C-5 were different each other [**3**: $\delta_H$ 2.54 (dd, $J_{5,10}$= 13.0 Hz), **5**: 3.91 (br s, $J_{5,10}$ < 6 Hz)]. The paramagnetic shift observed in **5** was obviously attributable to a strong anisotropic effect of carbonyl groups at C-20, C-6 and C-18, indicating that a hydrogen at C-5 and a C-9−C-20 bond are in a *cis* relationship. In nOe experiments, irradiation of H-5α at δ 3.91 caused a positive enhancement at the signals of H-10α and H-7α, and the latter signal was additionally enhanced by irradiation

of Me-8α at δ 1.04, which supported that the hydrogen at C-5 was in an α-configuration. To confirm the structure proposed above, a single-crystal X-ray diffraction was undertaken. The X-ray molecular model is shown in Figure 1, which exhibited that rings A and B are in a chair conformation and they are *cis*-fused,

Table 1 $^1$H Nmr spectral data for **3** and **5**-**8** ($CDCl_3$, 400 MHz, δ values in ppm, *J* values in Hz)

| | **3** | **5** | **6** | **7** | **8** |
|---|---|---|---|---|---|
| 4α-H | 2.18 (m) | 2.20 (m) | 2.18 (m) | 2.17 (m) | overlapped |
| 5α-H | | 3.91 (br s)[a] | 3.90 (br s)[a] | | |
| 5β-H | 2.54 (dd, 13.0)[b] | | 2.53 (dd, 13.0)[b] | overlapped | |
| 7-$H_A$ | 2.66 (dd, 13.7, 5.7) | 3.12 (dd, 14.1, <2) | 3.12 (dd, 14.1, <2) | 2.65 (dd, 13.7, 5.4) | 2.67 (dd, 13.7, 5.4) |
| 7-$H_B$ | 2.20 (dd, 13.7, 2.6) | 2.27 (dd, 14.1, 5.3) | 2.26 (dd, 14.1, 5.2) | 2.22 (dd, 13.7, 2.6) | 2.17 (dd, 13.7, 2.6) |
| 8β-H | 2.27 (m) | 2.18 (m) | 2.17 (m) | 2.27 (m) | 2.27 (m) |
| 11-$H_A$ | 3.14 (dd, 13.6, 8.8) | 2.58 (dd, 13.8, 6.5) | 2.55 (dd, 13.8, 6.6) | 3.13 (dd, 13.6, 8.7) | 3.16 (dd, 13.7, 8.7) |
| 11-$H_B$ | 2.34 (dd, 13.6, 6.2) | 2.35 (dd, 13.8, 10.3) | 2.33 (dd, 13.8, 10.3) | 2.34 (dd, 13.6, 6.2) | 2.33 (dd, 13.7, 6.2) |
| 12α-H | 5.48 (dd, 8.8, 6.2) | 5.45 (dd, 10.3, 6.5) | 5.43 (dd, 10.3, 6.6) | 5.48 (dd, 8.7, 6.2) | 5.48 (dd, 8.7, 6.2) |
| 14-H | 6.41 (m) | 6.43 (m) | 6.41 (m) | 6.41 (m) | 6.40 (m) |
| 15-H | 7.47 (m) | 7.46 (m) | 7.44 (m) | 7.47 (m) | 7.47 (m) |
| 16-H | 7.47 (m) | 7.51 (m) | 7.49 (m) | 7.47 (m) | 7.47 (m) |
| 17-Me | 1.12 (d, 7.3) | 1.06 (d, 6.8) | 1.04 (d, 6.3) | 1.12 (d, 7.3) | 1.11 (d, 7.1) |
| COOMe | 3.70 (s) | 3.64 (s) | | | |
| NH | | | | 5.98 (br s) | |
| NH | | | | 5.76 (br s) | |

[a] $J_{5,10} < 6$ Hz, [b] $J_{5,10}$

whereas ring C (C-9, C-11, C-12, C-20 and O-20) is in a semiboat conformation.

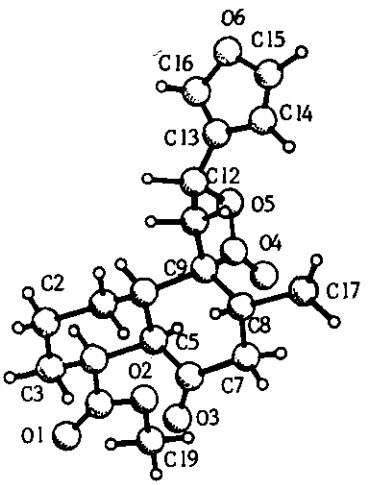



Figure 1 The X-ray model of compound (**5**)

When **1** was treated with *t*-BuOK in *t*-BuOH, an amorphous product (**6**) was obtained in 70% yield. The structure was supposed to be a keto acid with a *cis*-clerodane skeleton by comparison of spectral data with

those of **5**. Methylation of **6** with diazomethane gave **5**. To the contrary, treatment of teucvin (**2**) under the same conditions as **1** afforded a keto ester (**4**).

To examine the hydrolytic effect of other basic reagents on lactone moieties, **1** was treated with 0.5N NaOH in 50% aqueous MeOH to give an amorphous product (**7**) in an almost quantitative yield. The product (**7**) was characterized as a keto acid derivative on the basis of the ir (3153, 1739 and 1709 $cm^{-1}$) and nmr [$\delta_H$ 4.70 (br s, OH), $\delta_C$ 207.5 (s)] spectral data, and its stereochemistry was proved to be identical with that of **3**. On the other hand, a product (**8**) was formed by aminolysis in 72% yield when **1** was treated with an aqueous $NH_4OH$. The spectral data of **8** clearly revealed an amide structure [ir: 3440, 3360, 3200, 1710, 1660 $cm^{-1}$; $^1H$ nmr: $\delta_{NH}$ 5.98 (br s) and 5.76 (br s)], and the stereochemistry was depicted **8** by reason of nmr spectral identity based on the skeleton with **3** and **7**.

Some *nor*-clerodane derivatives described above were tested antifeedant activity against the larvae of *Leucania separata*. The results are shown in Table 2 along with the activity of ajugacumbin A[11] and **1**, and revealed that the structure modification caused a noticeable increase in the activity as found in **7** or decrease in **6** and **8**. A carboxyl group at C-4 will play a role in increasing the antifeedant activity. The results also suggested that the chemical transformation of naturally occurring *neo*-clerodane derivative is useful clue to discovery of a potent antifeedant substance.

Table 2 Antifeedant activity of test compounds against the larvae of *Leucania separata* Walker in non-choice leaf discs methods[3] using fresh leaf of *Zea mays* L.

| Compounds | Antifeedant Index[a] |
|---|---|
| ajugacumbin A[b] | 73.7 |
| **1** | 52.6 |
| **3** | 42.1 |
| **6** | 31.6 |
| **7** | 100.0 |
| **8** | 15.8 |

[a]Antifeedant index [(C-T)/(C+T)]% obtained when the larvae were exposed to discs treated with 1000 ppm of the test compounds, three replications per compound. [b]Our previous bioassays showed that the lowest effective concentration of ajugacumbin A is 50 ppm against the larvae of *Pareba vesta* Fabricius.[11]

## EXPERIMENTAL

Melting points are uncorrected. $^1H$ and $^{13}C$ nmr spectra were measured on a JNM-GX400 spectrometer. Chemical shifts are given in δ values (TMS as internal standard). Ir spectra were recorded with a Perkin-

Elmer 983 spectrophotometer. Eims were recorded with a JEOL JMS D-300 high resolution mass spectrometer. Petroleum ether refers to a fraction bp 60-90°C. Starting material (**1**) was available from our previous work.[7]

**Preparation of keto esters (3 and 5) from teucvidin (1)**[8,10]

Teucvidin (**1**) (1 g, 3.1 mmol) was added in MeOH (100 ml) and the mixture was heated under reflux for 20 h after addition of $Na_2CO_3$ (1 g, 9.4 mmol). After filtration, the filtrate was concentrated *in vacuo* and $H_2O$ (20 ml) was added to the solution. The mixture was neutralized with 1% HCl, and extracted with $CH_2Cl_2$. Usual workup of the extract gave a crude material, which was chromatographed on silica gel column eluted with petrol ether-EtOAc (3:1) to give amorphous products (**3**) (800 mg) and (**5**) (80 mg). Rf values of **3** and **5** were 0.39 and 0.42, respectively (silica gel plate, petrol ether : EtOAc = 3 : 1). **3**: Colorless squares, mp. 140-141°C (MeOH). Ir $\nu_{max}$ (KBr, $cm^{-1}$): 1760, 1720, 1600, 1500, 1440, 1180, 870. Eims *m/z* (rel. int.): 360 ($M^+$, 66), 329 (28), 301 (16), 300 (11), 266 (28), 234 (65), 221 (13), 220 (81), 206 (33), 187 (17), 178 (72), 161 (44), 150 (28), 147 (24), 134 (27), 121 (38), 105 (27), 95 (65), 94 (100) 91 (39), 81 (51), 69 (62). $^1H$ Nmr: Table 1. **5**: Colorless squares, mp 193-194°C (MeOH). Ir $\nu_{max}$ (KBr, $cm^{-1}$): 1738, 1696, 1597, 1506, 1203, 1020, 872. Eims *m/z* (rel. int.): 360 ($M^+$, 39), 329 (35), 328 (20), 300 (15), 266 (47), 234 (89), 220 (37), 206 (40), 178 (57), 161 (43), 150 (39), 121 (36), 105 (24), 95 (36), 94 (56), 81 (44), 69 (50), 57 (41), 42 (100). $^1H$ Nmr: Table 1. $^{13}C$ Nmr ($CDCl_3$) δ: 16.9 (q, C-17), 23.1 (t, C-2), 24.9 (t, C-1), 25.5 (t, C-3), 32.8 (d, C-8), 36.5 (t, C-11), 42.3 (d, C-10), 43.3 (d, C-5), 45.4 (t, C-7), 47.4 (d, C-4), 51.6 (s, C-9), 51.7 (q, COO<u>Me</u>), 70.9 (d, C-12), 108.1 (d, C-14), 123.9 (s, C-13), 140.0 (d, C-16), 144.2 (d, C-15), 173.7 (s, C-18), 176.9 (s, C-20), 208.8 (s, C-6). *Anal.* Calcd for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.70; H, 6.58.

**Crystallographic data of 5**

Compound (**5**) was recrystallized from MeOH for X-ray analysis. Orthorhombic system, space group $P2_12_12_1$, cell dimensions are a= 8.703(4), b= 13.833(7), c= 15.379(20) Å, Z= 4, V= 1851.65(28) $Å^3$, Dc= 1.29 g/cm. Intensity data were collected in the range of $0 < \theta < 57$ by an R3m/E four-circle diffractometer, $CuK_\alpha$ radiation (graphite-monochromated), ω scan, and 1268 independent reflections were recorded; 682 with $I \geq 3\sigma$ (I) were considered as observed. The molecular structure was solved by direct methods with SHELXS-86 programs. Twenty-four atoms were located from E map, and refined by full-matrix least-squares methods with anisotropic thermal parameters for the non-hydrogen atoms. R= 0.0795.

**Preparation of a keto acid (6) from 1**

Potassium (13 mg, 0.33 mmol) was dissolved in *t*-BuOH (5 ml) under nitrogen atmosphere with warming. To the solution was added **1** (50 mg, 0.15 mmol), and the mixture was heated at 115°C for 40 min under nitrogen. After acidified with 10% HCl, the mixture was extracted with $CH_2Cl_2$. Usual workup gave a crude reactant (35 mg), which was chromatographed on silica gel column eluted with $CHCl_3$-MeOH (5 : 1) to give an amorphous product (**6**). **6**: mp 139-141°C. Ir $\nu_{max}$ (KBr, $cm^{-1}$): 1751, 1709, 1502, 1450, 1180, 875. Eims *m/z* (rel. int.): 346 ($M^+$, 32), 328 (23), 300 (9), 252 (19), 234 (36), 220 (49), 206 (26), 202 (17), 187 (14), 178 (58), 161 (40), 147 (19), 134 (22), 121 (37), 109 (18), 105 (22), 95 (57), 94 (100). $^1H$ Nmr: Table 1. $^{13}C$ Nmr($CDCl_3$) δ: 16.9 (q, C-17), 22.8 (t, C-2), 24.8 (t, C-1), 25.5 (t, C-3), 33.0 (d, C-8), 36.5 (d, C-11), 43.3 (d, C-5), 42.2 (d, C-10), 45.4 (t, C-7), 47.3 (d, C-4), 51.6 (s, C-9), 70.8 (d, C-12), 108.1 (d, C-14), 124.0 (s, C-13), 140.0 (d, C-16), 144.2 (d, C-15), 176.9 (s, C-20), 178.7 (s, C-18), 208.8 (s, C-6). High resolution eims *m/z* 346.1461 (Calcd 346.1416 for $C_{19}H_{22}O_6$). Compound (**6**) (10 mg) was methylated with diazomethane to yield a crude product, which was recrystallized from MeOH to give colorless squares (**5**) (8 mg).

**Preparation of a keto acid (7) from 1**

Teucvidin (**1**) (30 mg, 0.10 mmol) was dissolved in 0.5N NaOH in 50% aqueous MeOH (2 ml), and the mixture was allowed to react at 70°C for 15 h, and then neutralized with 0.5N HCl. The resulting mixture was extracted with $CH_2Cl_2$ and the extract was treated as usual to give a crystalline residue (25 mg) which was recrystallized from petrol ether-EtOAc (1 : 1) to achieve **7** (18 mg). **7**: mp 213-214°C. Ir $\nu_{max}$ (KBr, $cm^{-1}$): 3153, 1739, 1710, 1606, 1501, 1457, 1200, 876, 797. Eims *m/z* (rel. int.): 346 ($M^+$, 55), 328 (33), 300 (10), 252 (21), 234 (37), 220 (75), 206 (24), 202 (25), 187 (18), 178 (72), 161 (39), 134 (26), 121 (42), 109 (19), 105 (19), 95 (63), 94 (100), 91 (28), 82 (31), 81 (36), 77 (20), 69 (62). $^1H$ Nmr: Table 1. $^{13}C$ Nmr ($CDCl_3$) δ: 15.2 (q, C-17), 24.6 (t, C-2), 27.8 (t, C-1), 29.3 (t, C-3), 37.8 (d, C-8), 40.3 (t, C-11), 41.7 (d, C-5), 42.0 (d, C-10), 45.2 (t, C-7), 50.8 (d, C-4), 51.7 (s, C-9), 71.6 (d, C-12), 107.9 (d, C-14), 125.8 (s, C-13), 139.2 (d, C-16), 144.4 (d, C-15), 177.7 (s, C-20), 178.7 (s, C-18), 207.5 (s, C-6). *Anal.* Calcd for $C_{19}H_{22}O_6$: C, 65.88; H, 6.40. Found: C, 66.03; H, 6.51. Compound (**7**) (13 mg) was methylated with diazomethane to give a crude product, which was recrystallized from MeOH to yield colorless squares (**3**) (9 mg).

**Preparation of 8 from 1**

A mixture of **1** (250 mg, 0.76 mmol) and 25% $NH_4OH$ (25 ml) in MeOH (25 ml) was stirred for 30 h at 50°C, concentrated *in vacuo*, and then extracted with $CH_2Cl_2$. The extract was treated as usual to give a crude mixture of products (260 mg), which was chromatographed on silica gel column eluted with EtOAc to yield a colorless amorphous powder (**8**) (180 mg). **8**: mp 121-124°C. Ir $\nu_{max}$ (KBr, $cm^{-1}$): 3440, 3360, 3200, 1760, 1710, 1660, 1600, 1500, 1440, 1180, 880. Eims *m/z* (rel. int.): 345 ($M^+$, 3), 329 (8), 328 (35), 300 (5), 234 (100), 233 (24), 206 (45), 178 (15), 161 (30), 150 (15), 95 (27), 94 (39), 81 (22). *Anal.* Calcd for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06. Found C, 65.74; H; 6.68; N, 4.23.

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