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# HYDROXYGRINDELANE DERIVATIVES BY MICROBIAL TRANSFORMATION

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**Key Word Index**—*Aspergillus niger*; *Penicillium brevicompactum*; biotransformation; methyl 3α-hydroxy-7α,8α-epoxygrindelate; methyl 3α-hydroxy-6,8(17)-dehydrogrindelate; methyl 3α-hydroxygrindelate.

**Abstract**—The microbial transformation of methyl 7α,8α-epoxygrindelate and 6,8(17)-dehydrogrindelic acid by cultures of *Aspergillus niger* and *Penicillium brevicompactum* produced the corresponding 3α-hydroxygrindelanes.

## INTRODUCTION

In continuation of our effort to produce hydroxygrindelanes with potential insecticidal activity, we have subjected methyl 7α,8α-epoxygrindelate and 6,8(17)-dehydrogrindelic acid to microbial transformation using *Aspergillus niger* X-172 and *Penicillium brevicompactum* 10418, respectively.

## RESULTS AND DISCUSSION

We reported earlier [1] the microbial transformation of grindelic acid (**3**) into 3α-hydroxygrindelic acid (**5**) in good yield. We now report that methyl 7α,8α-epoxygrindelate (**1**) and 6,8(17)-dehydrogrindelic acid (**7**) are transformed analogously into methyl 3α-hydroxy-7α,8α-epoxygrindelate (**2**) and 3α-hydroxy-6,17-dehydrogrindelic acid (**9**), respectively.

In the EIMS, **2** displayed an $M^+$ peak at *m/z* 366 (*m/z* 366.2400 by HRMS: $C_{21}H_{34}O_5$ requires 366.2406), 16 mass units higher than that of **1**, and a mass spectral pattern very similar to that of **1**. This increment in the $M^+$ of 16 mu together with the presence of minor but diagnostic peaks at *m/z* 348 $[M-H_2O]^+$, 293 $[M-CH_2COOMe]^+$, 275 $[m/z\ 293-H_2O]^+$, 257 $[m/z\ 293-2H_2O]^+$, and a strong hydroxyl band (3450 $cm^{-1}$) in the IR (neat) spectrum of **2** clearly suggested that **1** had been hydroxylated. Since the *m/z* 226 species, which occurs as the base peak in **1** and **2**, includes the THF and B ring portions but lacks the A ring (Scheme 1), the hydroxyl group appeared to be in ring A. Supporting evidence came from a peak of appreciable intensity at *m/z* 252 (not observed in **1**), shifted from *m/z* 236 in **1** as required by the substitution of a hydroxyl group in ring A of **2**: its possible formulation is outlined in Scheme 1 (composition verified by HRMS). Since the peaks in the low mass region (below *m/z* 226) of the EIMS of **1** and **2** occur with same masses, and the same microorganism (*A. niger*) was used, the C-3 locus was favoured for the hydroxyl function in **2**.

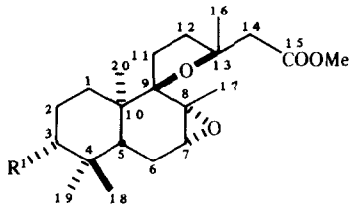
The $^1H$ (Table 1) and the $^{13}C$ (Table 2) NMR spectra confirmed the hydroxyl group in **2** to be in the 3 position, and showed it to be equatorial. The C-3 proton absorbs like that in **5** [1], and must be axial to have a coupling constant as large as 11.0 Hz. Compound **1** matches the NMR spectra of **2** except for the A ring peaks, which more closely match those of **5**.

The microbial transformation of **7** into its corresponding 3α-hydroxy derivative (**9**) was done on a mixture containing 75% of **3** and 25% of **7** (GC); we did not separate these substances from one another since they have virtually identical $R_f$ values on the chromatographic solvent systems used. Neither the biotransformation products **5** and **9** nor their corresponding methyl ester derivatives **6** and **10** were separated from one another for the same reason but their structures were evident from the $^1H$ NMR spectra of the 3:1 mixtures (Table 1) and received support from the EIMS of the mixture of **6** and **10**. Like diene **8**, the mixture exhibited little fragmentation but the peak at *m/z* 277 (loss of $CH_2CO_2Me$) and a *retro*-Diels–Alder fragment at *m/z* 210 [base, corresponding to *m/z* 196 (base) in 5], both derived from $M^+$ (*m/z* 350, not observed) of **6** and diagnostic peaks at *m/z* 348 ($M^+$, 3.2%) and 203 (19.4%), both shifted from *m/z* 332 ($M^+$) and 187 (base) in 8, clearly suggested that both **6** and **10** were hydroxylated. Since the *m/z* 187 ion (Fig. 1) in **8** includes the A and B rings, the hydroxyl group in **10** must be in ring A.

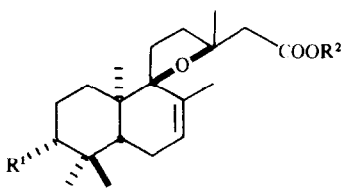
## EXPERIMENTAL

See references [2] for the analytical procedures, [1] for the description of microorganism and fermentation procedure and [3] for the GC analysis. A sample of methyl 7α,8α-epoxygrindelate (**1**) and of grindelic acid (**3**)-6,8(17)-dehydrogrindelic acid (**7**) mixture (3:1) were obtained by previously published procedure [4].

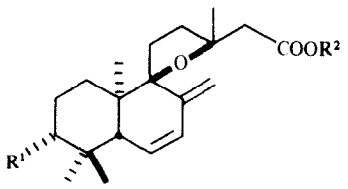
*Methyl 3α-hydroxy-7α,8α-epoxygrindelate* (**2**). The *A. niger* fermentation product (100 mg) was separated into $Et_2O$ soluble and insoluble fractions. From the $Et_2O$-soluble fr. (78 mg), **2**



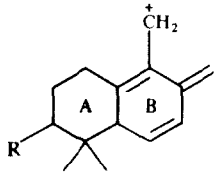
| | R |
|---|---|
| **1** | H |
| **2** | OH |



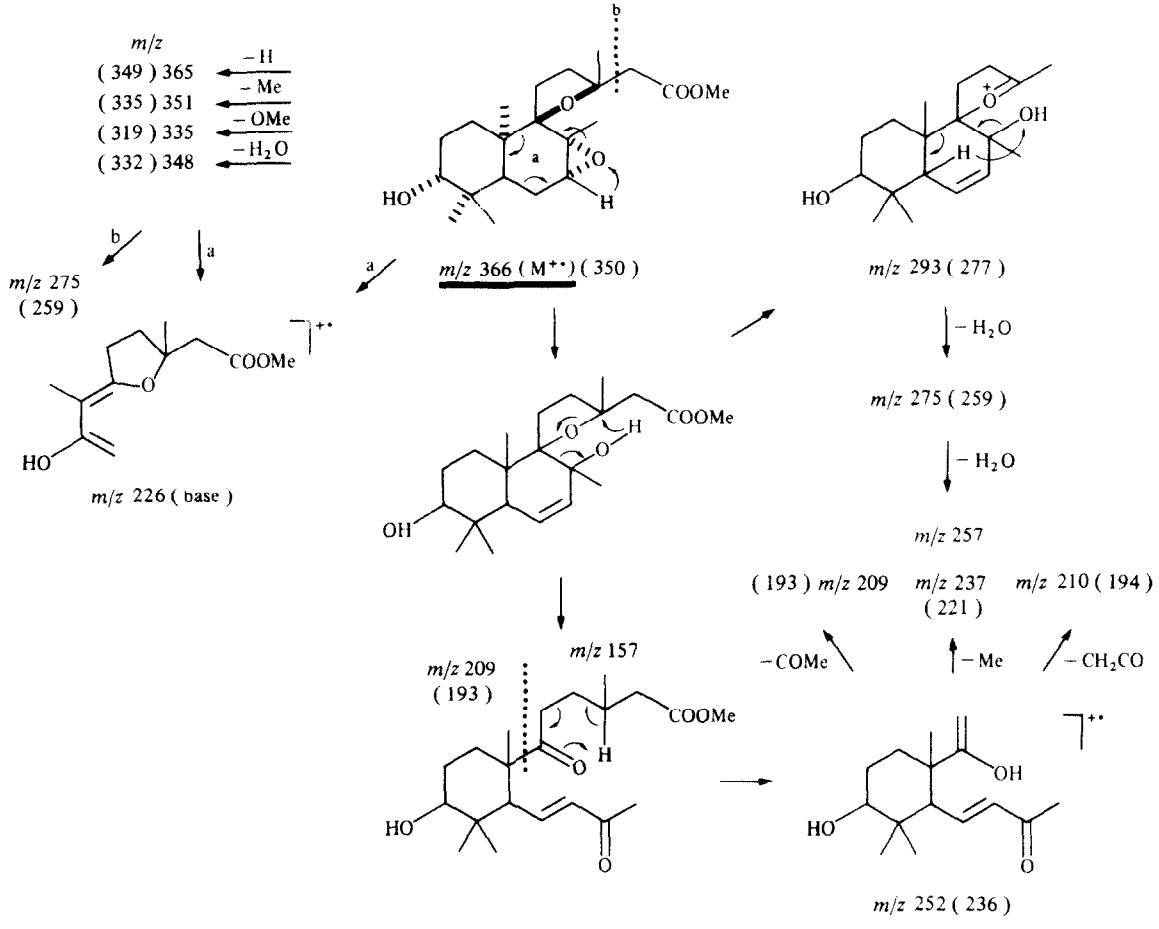
| | $R^1$ | $R^2$ |
|---|---|---|
| **3** | H | H |
| **4** | H | Me |
| **5** | OH | H |
| **6** | OH | Me |



| | $R^1$ | $R^2$ |
|---|---|---|
| **7** | H | H |
| **8** | H | Me |
| **9** | OH | H |
| **10** | OH | Me |



| | R | *m/z* |
|---|---|---|
| **8** | H | 187 ( base ) |
| **10** | OH | 203 |




Scheme 1. Diagnostic EIMS fragments of **2**. Figures in parentheses represent analogous ions in the EIMS of **1**.

Table 1. $^1$H NMR spectral data for compounds **1**, **2**, **5**, **6** and **8**–**10** (250 MHz, $CDCl_3$ with TMS as int. standard)

| H | 1 | 2 | 5[1] | 6 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|
| 3 | | 3.20 *dd*(11.0,4.3)* | 3.27 *dd*(10.8,4.3) | 3.29 *dd*(11.1,4.4) | | ‡ | 3.31 *dd*(10.8,4.3) |
| 5 | | | | | 2.43 *brs* | ‡ | 2.43 |
| 6 | | | | | 6.08 *dd*(10.1, 3.3) | 6.17 *dd*(10.2, 3.3) | 6.12 *dd*(10.1, 3.3) |
| 7 | 3.03 *m* | 3.07 *brs* | 5.65 *brs* | 5.52 *brs* | 5.80 *d*(10.1) | 5.84 *d*(10.2) | 5.78 *d*(10.1) |
| 14 | { 2.74 *d*(14.6) | 2.74 *d*(14.6) | 2.57 *d*(15.4) | 2.60 *d*(14.2) | 2.52 *d*(14.2) | | 2.52 (14.2) |
| | { 2.82 *d*(14.6) | 2.81 *d*(14.6) | 2.68 *d*(15.4) | 2.74 *d*(14.2) | 2.59 *d*(14.2) | ‡ | 2.58 *d*(14.2) |
| 16 | 1.34 *s* | 1.34 | 1.39 | 1.33 | 1.33 | 1.35 | 1.32 |
| 17 | 1.42 *s* | 1.42 | 1.79 *d*(1.4) | 1.77 *s* | 4.90, 5.06 | 5.04, 5.14 | 4.93, 5.07 |
| 18 | 0.87 *s*† | 0.96 | 0.99 | 0.98 | 0.99 | 1.11 | 1.11 |
| 19 | 0.86 *s*† | 0.85 | 0.89 | 0.87 | 0.85 | 0.83 | 0.82 |
| 20 | 0.83 *s*† | 0.85 | 0.84 | 0.82 | 0.72 | 0.75 | 0.72 |
| OMe | 3.67 *s* | 3.67 | | 3.66 | 3.62 | | 3.62 |

*Coupling constants in Hz in parenthesis.
†May be interchanged.
‡Obscured by peaks due to an impurity.

Table 2. $^{13}$C NMR spectral data for compounds **1**, **2** and **5** (22.63 MHz, $CDCl_3$ with TMS as int. standard)

| C | 1[6] | 2 | 5[1] |
|---|---|---|---|
| 1 | 31.8 | 29.7 | 30.8 |
| 2 | 18.3 | 27.2 | 27.0 |
| 3 | 41.7 | 78.1 | 78.5 |
| 4 | 33.1 | 38.6 | 38.8 |
| 5 | 37.2 | 37.2 | 42.3 |
| 6 | 22.9 | 22.5 | 23.8 |
| 7 | 61.5 | 61.9 | 128.4 |
| 8 | 59.0 | 59.0 | 133.2 |
| 9 | 88.3 | 88.1 | 92.1 |
| 10 | 39.8 | 39.6 | 40.5 |
| 11 | 29.1 | 29.2 | 27.7 |
| 12 | 37.5 | 37.4 | 39.2 |
| 13 | 81.4 | 81.5 | 81.2 |
| 14 | 47.1 | 47.0 | 47.4 |
| 15 | 171.9 | 171.8 | 172.4 |
| 16 | 26.7 | 27.2 | 26.8 |
| 17 | 23.1 | 22.9 | 21.0 |
| 18 | 32.5 | 27.2 | 27.6 |
| 19 | 22.5 | 15.4 | 15.1 |
| 20 | 16.5 | 16.6 | 16.8 |
| OMe | 51.2 | 51.3 | — |

(40 mg, single spot by TLC) was isolated by PLC [*n*-hexane–EtOAc–AcOH (20:20:3), single development]. Its IR (text), $^1$H NMR (Table 1), $^{13}$C NMR (Table 2) and mass (Scheme-1) spectra were in accord with the structure shown.

*Methyl 3α-hydroxy-6,8(17)-dehydrogrindelate* (**10**) *and methyl 3α-hydroxy-grindelate* (**6**). The *P. brevicompactum* fermentation product (240 mg) was extracted with $Et_2O$ and filtered. From the $Et_2O$-soluble filtrate (185 mg), the major spot (65.8 mg) was isolated by PLC [petrol–$Et_2O$–AcOH (5:5:1), single development] and methylated [5]. The methylated product (53 mg), when submitted to PLC [petrol–$Et_2O$–EtOAc (15:2:8), two developments], gave a TLC single spot fr (24.3 mg), shown by $^1$H NMR to be a mixture of **6** (70%) and **10** (30%). Its IR (text), $^1$H NMR (Table 1) and mass (text) spectra were in accord with the structures shown.

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