Pergamon

0031-9422(94)00856-6

*Phytochemistry*, Vol. 39 , No. 1, pp. 85–89, 1995

0031-9422/95 $9.50 + 0.00

# BIOTRANSFORMATION OF THE MONOTERPENOID, ROSE OXIDE, BY *ASPERGILLUS NIGER*

MITSUO MIYAZAWA,* KATSUYUKI YOKOTE and HIROMU KAMEOKA

Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Kowakae, Higasiosaka-shi, Osaka 577, Japan

(*Received in revised form* 6 *September* 1994)

**Key Word Index**—*Aspergillus niger*; monoterpenoid; biotransformation; ( − )-*cis*-rose oxide; ( − )-*trans*-rose oxide; ( − )-*cis*-9-hydroxy-7*E*-rose oxide; ( − )-*trans*-9-hydroxy-7*E*-rose oxide; ( − )-*cis*-7*E*-rose oxide-8-carboxylic acid; ( − )-*trans*-7*E*-rose oxide-8-carboxylic acid.

**Abstract**—The biotransformation of ( − )-*cis*-rose oxide and ( − )-*trans*-rose oxide has been carried out using the fungus *Aspergillus niger*. Each oxide was converted to the corresponding primary alcohol (9-hydroxy-7*E*-rose oxide) as the major product, some of which was further oxidized to the 8-carboxylic acid of the parent rose oxide. The structures of these metabolites have been established by spectral means.

## INTRODUCTION

The importance of monoterpenoids as components of flavours and perfumes is continually growing. The diastereoismeric rose oxides (*cis*-form, **1** and *trans*-form, **5**) found originally in rose oil [1] and geranium oil [2] are among the most appreciated ingredients in fine perfumery. The biotransformation of rose oxide has not been reported. We are investigating biotransformations from a pharmacological point of view. In our previous papers, we reported on the biotransformation of ( + )-fenchone [3], ( − )-α-bisabolol [4], 1,4-cineole [5] and lignan [6] by *Aspergillus niger*. This paper deals with the microbial oxidation of ( − )-*cis*-rose oxide (**1**) and ( − )-*trans*-rose oxide (**5**) to ( − )-*cis*-9-hydroxy-7*E*-rose oxide (**2**) and ( − )-*cis*-7*E*-rose oxide-8-carboxylic acid (**3**), ( − )-*trans*-9-hydroxy-7*E*-rose oxide (**6**) and ( − )-*trans*-7*E*-rose oxide-8-carboxylic acid (**7**), respectively, by the same organism.

## RESULTS AND DISCUSSION

*Aspergillus niger* was cultured as described previously [3–6]. On day 2 ( − )-*cis*-rose oxide (**1**) or ( − )-*trans*-rose oxide (**5**) was added to the medium and the cultures left for a further five days. The metabolites were then obtained by liquid–liquid extraction using dichloromethane.

The biotransformation of **1** for five days by *A. niger* resulted in the formation of the corresponding primary alcohol (*cis*-9-hydroxy-7*E*-rose oxide) (**2**) as major product. When the biotransformation period was reduced to two days, the starting material completely disappeared and a new metabolite, **3**, was obtained (Fig. 1). The biotransformation of **5** gave the corresponding primary alcohol (**6**) which, as in the case of **2**, was further oxidized to the secondary metabolic product **7** (Fig. 2). These products were not detected on TLC and GLC analysis of the culture dish of *A. niger* to which no substrate was fed, and of a mixture of **1** or **5** and the medium cultured for five days, respectively.

The pH value of the medium with each substrate and those of the medium without substrate varied between 3 and 7, respectively (Fig. 3). There was, therefore, a possibility that **2**, **3** and **6**, **7** were produced as a result of pH-dependent autoxidation reactions. However, on adding substrates **1** and **5** to the medium and changing the pH (3 to 7) artificially products **2** and **3**, and **6** and **7** could not be detected by TLC or GC. This result established that products **2** and **3**, and **6** and **7** were indeed true biotransformation products.

The structures of the metabolites were determined by comparison of their $^1$H, $^{13}$C NMR, IR and mass spectra with those of **1** and **5**. In the $^1$H NMR spectrum of **2**, $C_{10}H_{18}O_2$ (*m/z* 170), the signal of one of the terminal allylic methyl groups (C-9, C-10) in the original compound (**1**) was replaced by the signals assignable to a hydroxymethyl group ($\delta_H$ 3.98, $\delta_C$ 67.8), indicating that **2** was either the C-9 or C-10 hydroxylated derivative of rose oxide. The position of the hydroxy group was determined by comparison with the literature data for similar part structures [7]. Accordingly, based on the mass, IR, $^1$H and $^{13}$C NMR spectral data, the structure of **2** was elucidated to be ( − )-*cis*-9-hydroxy-7*E*-rose oxide.

The $^1$H and $^{13}$C NMR spectra of **6** resembled those of **5** except for the replacement of the signal of one of the

*Author to whom correspondence should be addressed.

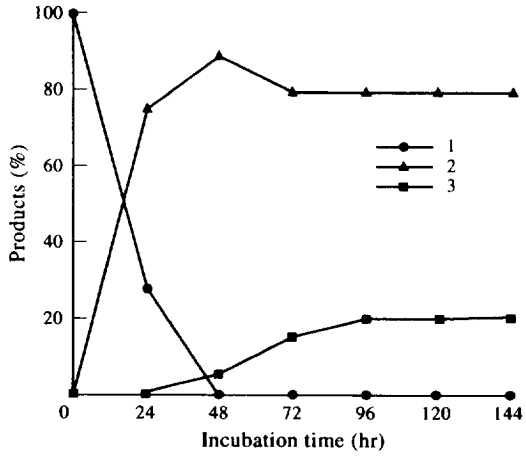



Fig. 1. Conversion of **1** to **2** and **3**.

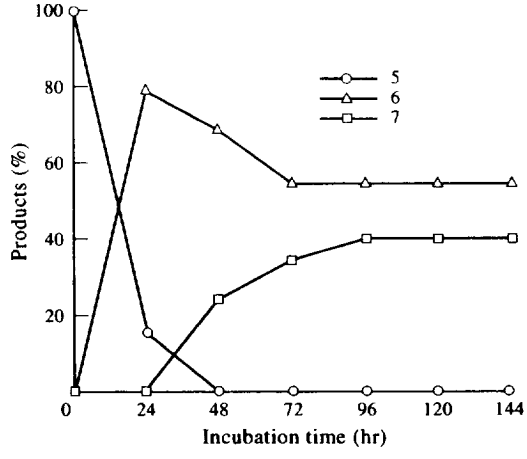



Fig. 2. Time course of use of (−)-*trans* rose oxide **5** and production of **6** and **7**.

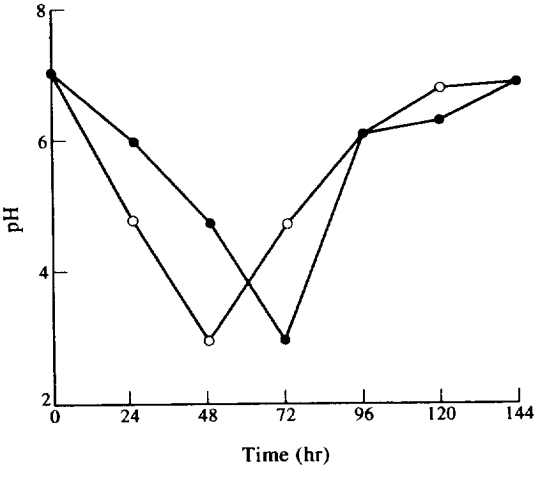



Fig. 3. pH changes in the medium with (●) and without (○) rose oxide.

Table 1. $^1$H NMR spectral data for compounds **1**–**4** (500 MHz, $CDCl_3$, TMS as int. standard)

| H | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 2 | 3.96 *ddd* (11.5, 8, 2) | 4.04 *ddd* (11.5, 8, 2) | 4.14 *ddd* (11, 8, 2) | 4.12 *ddd* (8, 8, 3.5) |
| 3 ax | 1.0 *ddd* (13, 11.5, 11.5) | 1.05 *ddd* (13, 11.5, 11.5) | — | — |
| 3 eq | 1.47–1.65 *m* | 1.5–1.68 *m* | — | — |
| 4 | 1.47–1.65 *m* | 1.5–1.98 *m* | — | — |
| 5 ax | 1.19 *dddd* (12.5, 12.5, 12.5, 5) | 1.24 *dddd* (12.5, 12.5, 12.5, 5) | 1.43 *dddd* (12.5, 12.5, 12.5, 5) | 1.42 *dddd* (12.5, 12.5, 12.5, 5) |
| 5 eq | 1.47–1.65 *m* | 1.5–1.68 *m* | — | — |
| 6 ax | 3.44 *ddd* (12.5, 12.5, 2) | 3.47 *ddd* (12.5, 12.5, 2) | 3.48 *ddd* (12.5, 12.5, 2) | 3.47 *ddd* (12.5, 12.5, 2) |
| 6 eq | 3.97 *ddd* (12.5, 12.5, 2) | 3.99 *ddd* (12.5, 5, 2) | 4.03 *ddd* (12.5, 5, 2) | 4.02 *ddd* (12.5, 5, 2) |
| 7 | 5.14 *d* (8) | 5.24 *d* (8) | 6.80 *d* (8) | 6.66 *d* (8) |
| Me-9 | 1.67 *d* (1.5) | — | — | — |
| $CH_2$-9 | — | 3.98 *s* | — | — |
| $CO_2$Me-9 | — | — | — | 3.73 *s* |
| Me-10 | 1.70 *d* (1.5) | 1.70 *d* (1.5) | 1.87 *d* (1.5) | 1.87 *d* (1.5) |
| Me-11 | 0.92 *d* (6.2) | 0.95 *d* (6.2) | 0.96 *d* (6.2) | 0.96 *d* (6.2) |

Coupling constants in Hz.

terminal allylic methyl groups (C-9, C-10) in **5** by the signal of a hydroxymethyl group ($\delta_H$ 4.0, $\delta_C$ 67.8). The position of hydroxy group was determined to be the same as in **2**. The structure of **6** was, therefore, formulated as ( − )-*trans*-9-hydroxy-7*E*-rose oxide, which is an isomer of **2**.

The secondary metabolites (**3** and **7**) from the acidic part were purified as their methylesters (**4** and **8**) following methylation with diazomethane. The IR and $^{13}$C NMR spectra of **4**, $C_{11}H_{18}O_3$ (*m/z* 198), showed the presence of a carboxyl group ($\nu_{max}$ 1718 cm$^{-1}$, $\delta_C$ 168.0) while the signal corresponding to one of the terminal allylic methyl groups (C-9, C-10) observed in **1** was absent from the $^1$H NMR spectrum. The signal of methyl group of $CO_2Me$ was also observed. From the chemical shift of the proton at C-7 ($\delta_H$ 6.66), **4** was determined to be the methyl ester of ( − )-*cis*-7*E*-rose oxide-8-carboxylic acid (**3**) formed by conversion of the C-9 methyl group of **1** to a carboxy group.

The spectral properties of **8** closely resembled those of **5**, apart from the presence of a carboxyl group ($\nu_{max}$ 1718 cm$^{-1}$, $\delta_C$ 168.3) which was not observed in **5**. In the $^1$H NMR spectrum of **8**, the signal of one of the terminal allylic methyl groups (C-9, C-10) was absent as in the case of **4**. This suggested that **8** was the isomer of **4**. The position of the carboxy group was determined by a comparison with the usual chemical shift of H-7 proton ($\delta$6.84), **8** was determined to be the methylester of ( − )-*trans*-7*E*-rose oxide-8-carboxylic acid (**7**). The $^1$H and $^{13}$C NMR data of compounds **1**–**8** are shown in Tables 1–3. As shown in Scheme 1, the biotransformation of the two diasteroisomeric rose oxides, **1** and **5**, gave the metabolic products **2** and **6**, respectively, in the first step and the corresponding 8-carboxylic acid in the second step. All of these products had *E*-configuration at the C-7/C-8 double bond. Thus the fungus *A. niger* oxidized the C-9 position of rose oxide diastereoselectively. The time courses for the conversion of **1** and **5** by *A. niger* are shown in Figs 1 and 2. The relative amount of each compound was calculated from the peak area on GC.

Table 2. $^1$H NMR spectral data for compounds **5**–**7** (500 MHz, $CDCl_3$, TMS as int. standard)

| H | **5** | **6** | **7** | **8** |
|---|---|---|---|---|
| 2 | 4.36 *ddd* (8, 8, 3.5) | 4.44 *ddd* (8, 8, 3.5) | 4.56 *ddd* (8, 8, 3.5) | 4.52 *ddd* (8, 8, 3.5) |
| 3 ax | 1.6 *ddd* (13, 8, 4.8) | 1.66 *ddd* (13, 8, 4.8) | — | — |
| 3 eq | 1.36 *dddd* (13, 6, 3.5, 1.2) | 1.38 *dddd* (13, 6, 3.5, 1.2) | — | |
| 4 | 2.02 *m* | 2.02 *m* | 1.8 *m* | 2.0 *m* |
| 5 ax | 1.76 *dddd* (13, 8, 4.8, 4.8) | 1.76 *dddd* (13, 8.5, 4.8, 4.8) | 1.76 *dddd* (13, 8, 4.8, 4.8) | 1.76 *dddd* (13, 8, 4.8, 4.8) |
| 5 eq | 1.24 *ddddd* (13, 5, 3, 3, 1.2) | 1.26 *ddddd* (13, 5, 3, 3, 1.2) | — | — |
| 6 ax | 3.69 *ddd* (12, 8.5, 3) | 3.72 *ddd* (12, 8.5, 3) | 3.7 *ddd* (12, 8.5, 3) | 3.7 *ddd* (12, 8.5, 3) |
| 6 eq | 3.75 *ddd* (12, 4.8, 3) | 3.76 *ddd* (12, 4.8, 3) | 3.75 *ddd* (12, 4.8, 3) | 3.75 *ddd* (12, 4.8, 3) |
| 7 | 5.28 *d* (8) | 5.56 *d* (8) | 6.98 *d* (8) | 6.84 *d* (8) |
| Me-9 | 1.69 *d* (1.5) | — | — | — |
| $CH_2$-9 | — | 4.0 *s* | — | — |
| $CO_2$Me-9 | — | — | — | 3.74 *s* |
| Me-10 | 1.72 *d* (1.5) | 1.70 *d* (1.5) | 1.88 *d* (1.5) | 1.88 *d* (1.5) |
| Me-11 | 1.06 *d* (7) | 1.07 *d* (7) | 1.06 *d* (6.2) | 1.06 *d* (6.2) |

Coupling constants in Hz.

## EXPERIMENTAL

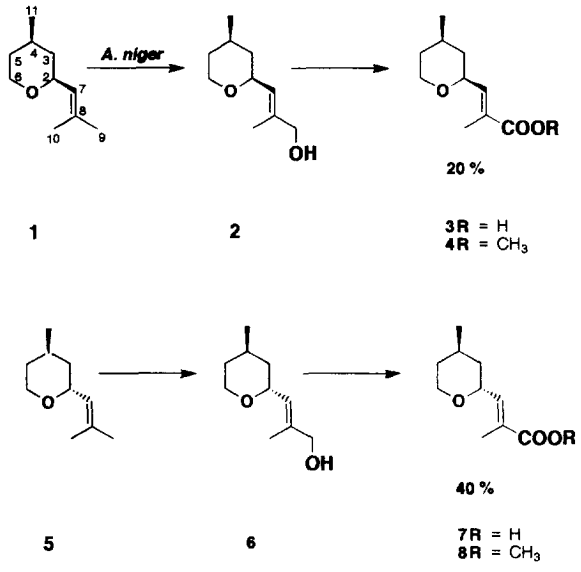
( − )-*cis*-Rose oxide (**1**) and ( − )-*trans*-rose oxide (**5**) were a gift from the Taiyou perfumery Co. Ltd. $^1$H and $^{13}$C NMR: 500 MHz with TMSi as int. standard; $^{13}$C NMR: 125 MHz with TMSi as int. standard; MS: 70 eV; GC: OV-1 (25 m × 0.25 mm) capillary column.

*Preculture of Aspergillus niger.* Spores of *A. niger* IFO 4414 (purchased from the Institute of Fermentation of Osaka) were inoculated into a plate containing 15 ml of the following medium: saccharose 15 g, glucose 15 g, polypeptone 5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KCl 0.5 g, $K_2HPO_4$ 1 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, $H_2O$ 1 l. Cultivation was arried out at 28° without shaking for 2 days.

*Addition of* ( − )-cis-*rose oxide* (**1**). After growth of *A. niger* for 2 days, 2.0 g of ( − )-*cis*-rose oxide (1) (15 mg/15 ml of medium) was added to the medium and the culture left for 6 days.

Table 3. $^{13}C$NMR spectral data for compounds **1–8** (125 MHz, $CDCl_3$, TMS as int. standard)

| C | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 2 | 74.6 | 74.3 | 74.9 | 74.8 | 69.0 | 68.8 | 69.7 | 69.5 |
| 3 | 40.8 | 40.4 | 38.9 | 39.1 | 38.1 | 37.9 | 37.0 | 37.3 |
| 4 | 30.3 | 30.1 | 30.0 | 30.0 | 25.7 | 24.8 | 24.9 | 24.9 |
| 5 | 34.4 | 34.2 | 34.1 | 34.1 | 32.4 | 32.3 | 32.5 | 32.5 |
| 6 | 67.8 | 67.8 | 67.8 | 67.8 | 62.1 | 62.2 | 62.3 | 62.3 |
| 7 | 126.4 | 126.1 | 128.3 | 128.8 | 125.3 | 125.4 | 127.4 | 128.0 |
| 8 | 135.0 | 137.8 | 143.9 | 141.7 | 135.5 | 138.1 | 143.2 | 141.0 |
| 9 | 25.6 | 67.8 | 172.9 | 168.3 | 24.9 | 67.8 | 172.9 | 168.3 |
| 10 | 18.3 | 14.0 | 12.6 | 12.9 | 18.2 | 13.9 | 12.5 | 12.8 |
| 11 | 22.3 | 22.2 | 22.2 | 22.2 | 19.1 | 19.5 | 19.6 | 19.5 |
| $CO_2Me$ | | | | 51.8 | | | | 51.8 |




Scheme 1. Metabolism of ( − )-*cis*-rose oxide and ( − )-*trans*-rose oxide.

*Purification of metabolites* **2** *and* **3**. At the end of the incubation period, the culture broth was filtered to remove the mycelial mat. The filtrate was acidified to pH 2 and extracted continuously with $CH_2Cl_2$ for 72 hr. The organic layer was dried ($Na_2SO_4$) and then the solvent was evapd to give an oily residue (1.2 g). The extract was dissolved in $CH_2Cl_2$, washed with 5% $NaHCO_3$ soln and sepd in the usual manner. For purification of metabolite **2**, the neutral fraction was purified by silica gel CC with n-hexane containing an increasing concentration of $Et_2O$. This gave 0.11 g **2**. For purification of metabolite **3**, the acidic fraction was methylated with $CH_2N_2$ in the usual way, and purified by silica gel CC with the same solvent system to give **3** (0.022 g).

*Purification of metabolites* **6** *and* **7**. The metabolic products **6** (0.14 g) and **7** (0.048 g) were obtained in the same way as **2** and **3**.

*Biotransformation of* ( − )-cis-*rose oxide* (**1**) *and* ( − )-trans-*rose oxide* (**5**). Fermentation of **1** (2.0 g) with *A. niger* gave, after 5 days, ( − )-*cis*-9-hydroxy-7*E*-rose oxide (**2**) (110 mg) and ( − )-*cis*-7*E*-rose oxide-8-carboxylic acid (**3**) (22 mg), while ( − )-*trans*-7*E*-rose oxide (**5**) (2.2 g) gave ( − )-*trans*-9-hydroxy-7*E*-rose oxide (**6**) (140 mg) and ( − )-*trans*-7*E*-rose oxide-8-carboxylic acid (**7**) (48 mg).

*Compound* **1**. Oil. MS *m/z* (rel. int.): 154 $[M]^+$ (11.5), 139 (100), 83 (31.7), 69 (81.6), 55 (37.4), 41 (41.3); $[\alpha]_D^{20}$ − 69.1° ($CHCl_3$; *c* 0.82); IR $\nu_{max}$ $cm^{-1}$: 2951, 2924, 2837, 2801, 1678, 1456, 1377, 1257, 1169, 1092, 1080, 1053, 982, 881, 839; NMR: Tables 1 and 3.

*Compound* **2**. Oil. MS *m/z* (rel. int.): 170 $[M]^+$ (9.6), 155 (14.4), 139 (73.0), 115 (29.8), 97 (10.6), 83 (17.3), 69 (100), 55 (71), 41 (11.5); $[\alpha]_D^{20}$ − 33.2° ($CHCl_3$; *c* 0.42); IR $\nu_{max}$ $cm^{-1}$: 3409, 2954, 2942, 2883, 2871, 1678, 1455, 1381, 1250, 1172, 1068, 1011, 983, 885, 838; NMR: Tables 1 and 3.

*Compound* **4**. Oil. MS *m/z* (rel. int.): 198 $[M]^+$ (7.7), 183 (17.3), 166 (45.1), 139 (54.7), 123 (40.3), 113 (34.6), 97 (49.0), 81 (15.4), 69 (100), 55 (63.4), 41 (61.4); $[\alpha]_D^{20}$ − 32.6° ($CHCl_3$; *c* 0.12); IR $\nu_{max}$ $cm^{-1}$: 2951, 2924, 2868, 2841, 1718, 1658, 1456, 1376, 1255, 1168, 1059, 978, 885, 851; NMR: Tables 1 and 3.

*Compound* **5**. Oil. MS *m/z* (rel. int.): 154 $[M]^+$ (10.6), 139 (100), 83 (30.7), 69 (83.5), 55 (38.4), 41 (41.3); $[\alpha]_D^{20}$ − 9.5° ($CHCl_3$; *c* 0.74); IR $\nu_{max}$ $cm^{-1}$: 2954, 2940, 2853, 2830, 1678, 1458, 1381, 1250, 1172, 1083, 1053, 1029, 988, 881, 833; NMR: Tables 2 and 3.

*Compound* **6**. Oil. MS *m/z* (rel. int.): 170 $[M]^+$ (9.6), 155 (67.3), 139 (64.4), 115 (27.4), 97 (13.4), 83 (28.8), 69 (100), 55 (59.6), 42 (39.4); $[\alpha]_D^{20}$ − 21.3° ($CHCl_3$; *c* 0.22); IR $\nu_{max}$ $cm^{-1}$: 3409, 2954, 2942, 2883, 2871, 1678, 1455, 1381, 1250, 1172, 1068, 1011, 983, 885, 838; NMR: Tables 2 and 3.

*Compound* **8**. Oil. MS *m/z* (rel. int.): 198 $[M]^+$ (7.7), 183 (21.1), 166 (50.9), 139 (56.6), 123 (44.2), 113 (43.2), 97 (55.7), 81 (7.3), 69 (100), 55 (73.0), 41 (63.4); $[\alpha]_D^{20}$ − 24.8° ($CHCl_3$; *c* 0.24); IR $\nu_{max}$ $cm^{-1}$: 2954, 2942, 2883, 2871, 1718, 1658, 1455, 1381, 1255, 1172, 1058, 980, 885, 835; NMR: Tables 2 and 3.

*Acknowledgements*—We are grateful to the Taiyou Co. Ltd (Osaka, Japan) for supplying the samples. We are

also grateful to the Environmental Science Institute, Kinki University, for financial supprt. This study was also supported by a Grant-in-Aid for Science Research from the Japan Private School Promotion (1994).

## REFERENCES

1. Seidel, C. F. and Stoll, M. (1959) *Helvetica.* **42**, 1830.
2. Naves, Y. R., Lamparsky, D. and Ochsner, P. (1961) *Bull. Soc. Chim. Fr.* 645.
3. Miyazawa, M., Yamamoto, K., Noma, Y. and Kameoka H. (1990) *Chem. Express* **5**, 407.
4. Miyazawa, M., Funatsu, Y. and Kameoka, H. (1990) *Chem. Express* **5**, 589.
5. Miyazawa, M., Noma, Y., Yamamoto, K. and Kameoka, H. (1991) *Chem. Express* **6**, 771.
6. Miyazawa, M., Kasahara, H. and Kameoka, H. (1993) *Phytochemistry* **34**, 1501.
7. Arfmann, H.-A., Abraham, W.-R. and Kieslich, K. (1988) *Biocatalysis* **2**, 59.