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# MODELS OF RETINOID METABOLISM: MICROBIAL BIOTRANSFORMATION OF α-IONONE AND β-IONONE

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ABSTRACT.—Retinoic acid [**1**] and its analogues (retinoids) have recently generated interest as possible chemopreventive agents, but **1** is rapidly metabolized to many known as well as unknown products. Recently, microbial models have been employed for the study of mammalian metabolism. In this study two fungi, *Aspergillus niger* ATCC 16888 and *Cunninghamella blakesleeana* ATCC 8688a, were found to biotransform β-ionone [**2**] and α-ionone [**8**] and may serve as models for the mammalian metabolism of retinoic acids. *A. niger* yielded oxidized metabolites, whereas *C. blakesleeana* gave products most of which were both oxidized and reduced. Methods developed here should prove amenable to studies utilizing **1** as the substrate for biotransformation.

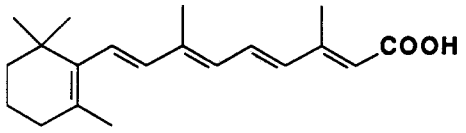
Vitamin A (retinol) exerts a number of biological effects in higher animals. all-*trans*-Retinoic acid [**1**], an oxidized metabolite of retinol, is capable of supporting the functions of retinol in the maintenance of normal growth and epithelial cell differentiation (1). Recently, **1** and its analogues have generated interest as agents useful in the treatment of skin disorders (2) and as possible cancer chemopreventive or chemotherapeutic agents (3). Although the mechanism of action of **1** remains unknown, several plausible theories have been put forth (4).

Studies of the metabolism of retinoic acid may provide insight into the biological action of this compound in animals. It has been suggested that retinol must be metabolized to retinoic acid for the maintenance of epithelial cell differentiation (5). The question of whether further metabolism to more active forms of the vitamin is important remains unanswered, although some evidence suggests that retinoic acid is the functional form (6).

However, some metabolites of retinoic acid have shown full biological activity. These metabolites include retinoyl-β-glucuronide (7) and 13-*cis*-retinoic acid (8). Other known metabolites of **1** include 5,6-epoxy- and 4-hydroxyretinoic acid, but these are relatively inactive in promoting differentiation of epithelial cells (8). Recently, several other metabolites of retinoic acid have been isolated from rats receiving physiological doses of retinoic acid (9). The structure and biological activity of many of these metabolites remain unknown (10). Therefore, the ultimate functional form of vitamin A necessary for epithelial differentiation may still remain to be discovered.

Microbial models have proven to be useful tools in the study of mammalian metabolism. The same types of metabolic reactions found in mammals may also occur in microorganisms. Phase I and phase II biotransformations, such as allylic hydroxylations, reductions, and conjugations, have been observed (11). Microbial models have been used to study metabolism of various xenobiotics, including steroids (12), cannabinoids (13,14), and alkaloids (15).

We have recently developed methods for the microbial transformation of β-ionone [**2**] and α-ionone [**8**]. These readily available natural products serve as suitable models for the metabolism of **1** for a number of reasons. The ionones have the advantage of higher solubility in aqueous media, lower toxicity, and greater chemical stability. The similarity in the trimethylcyclohexene rings of **1** and **2** is obvious, whereas **8** is structurally similar to α-retinoic acid [**12**]. This retinoid **12** has shown vitamin A-like activity in various assays (8,16).

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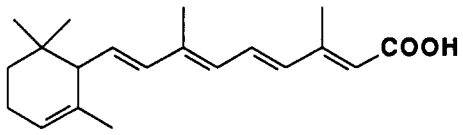


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We report in this communication our studies of the biotransformation of **2** and **8** by two fungi, *Aspergillus niger* ATCC 16888 and *Cunninghamella blakesleeana* ATCC 8688a. Results of these studies may provide insight into the possible identity of the unknown metabolites of **1**. The stereochemistry of many of the metabolites of **1** also remains unknown. Information about any stereospecificity to the metabolic reactions undergone by **1** might prove helpful in the identification of the mechanism of action of **1**. Stereochemical information could also assist in refining the design of analogues of **1** to be used for these types of studies (17).

## RESULTS AND DISCUSSION

Based on previous studies utilizing **1** (8) and **2** (18) as substrates, likely biotransformation products of **2** were synthesized for use as tlc standards to aid in preliminary identification. The synthetic procedures for the production of 4-hydroxy-β-ionone [**3**] and 4-oxo-β-ionone [**4**] have been previously reported by our laboratory (19). Oxidation of **2** with *m*-chloroperbenzoic acid provided 5,6-epoxy-β-ionone [**13**] quantitatively.

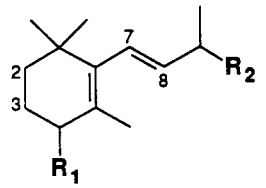
Initially, sixteen microorganisms were screened using a two-stage culture method (11) in order to discover those strains that afforded products of greater polarity than the substrate ionones, because most of the known metabolites of **1** are more polar than the parent compound. Two organisms were chosen for further study: *A. niger* and *C. blakesleeana*. Some of the products obtained from these organisms possessed chromatographic properties on Si gel tlc similar to those of reference **3**, **4**, and **13**.

A time course study of the metabolism of **2** was conducted using both organisms; only *A. niger* was utilized in the case of **8**. Cultures were harvested and metabolites assayed by tlc at various times up to 7 days after addition of substrate to the flasks. The apparent yields of total polar metabolites increased with longer incubation times.

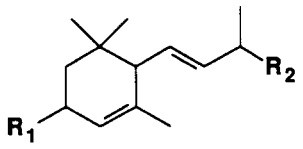
Chromatographic purification on Si gel using $Me_2CO/CH_2Cl_2$ or $EtOAc/C_6H_{14}$ mixtures as the solvent systems afforded the products shown in Table 1. Product structures were assigned by comparison with available synthetic standards or through the use of spectroscopic techniques. Many of the products isolated showed a significant optical rotation suggesting stereoselective metabolism of **2** and **8**.

Recently, Haag *et al.* (20) have established the absolute configuration of the enantiomers of **3** via their stereoselective synthesis from (*S*)-α-ionone. From these studies, (+)- and (−)-**3** have been assigned the *S* and *R* configuration, respectively. Compound **3** produced in this study by *A. niger* shows a specific rotation of −5.9° when measured under the conditions of Haag *et al.* (20). This observed rotation, therefore, allows us to estimate that an 82% enantiomeric excess of the *R* isomer of **3** is produced by allylic hydroxylation of **2** by *A. niger*.

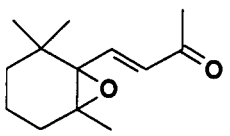
Independent confirmation of this estimated optical purity was obtained via the



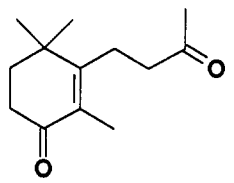

**2** $R_1$=H, $R_2$= =O
**3** $R_1$=OH, $R_2$= =O
**4** $R_1$= =O, $R_2$= =O
**5** $R_1$=camphanoyloxy, $R_2$= =O
**6** $R_1$= =O, $R_2$=OH




**8** $R_1$=H, $R_2$= =O
**9** $R_1$= =O, $R_2$= =O
**10** $R_1$=OH, $R_2$= =O
**11** $R_1$=$R_2$=OH




**13**




**7**

preparation of diastereomeric esters of **3** using the *R*-(−)-camphanic acid chloride method originated by Gerlach (21). Camphanoylation of synthetic racemic **3** readily produced an equal mixture of the *R,R* and *R,S* diastereomeric esters. An hplc system similar to that of Haag *et al.* (20) was developed that clearly resolved the two diastereomers. When acylation was repeated on the biosynthetic (−)-**3**, we observed, as did Haag, that the *R,R* diastereomeric ester had the longer retention time on hplc. Furthermore, the *R,R* and *R,S* diastereomers were present in a 6.7:1 ratio that corresponds to the production of a 74% enantiomeric excess of *R*(−)-**3** by *A. niger*. This observed optical purity is in remarkably good agreement with that estimated above from the specific rotation.

The enantiomeric composition of biosynthetic **3** was also estimated using the method of Dale *et al.* (22). The analysis of the 250-MHz $^1$H-nmr spectrum for the α-methoxy-α-trifluoromethylphenylacetate (MPTA) ester of biosynthetic **3** revealed two resonances for the 5-Me protons at δ 1.51 and 1.69. When these resonances were compared to those obtained for the same group in the MPTA ester of racemic **3** they were found to be identical. The area under these resonances showed the expected 1:1 ratio for the MPTA ester of the racemate but showed a 6.5:1 *R/S* ratio for the ester of biosynthetic **3**. Because this ratio compares favorably with that obtained utilizing hplc for the

TABLE 1. Isolates from the Biotransformation of **2** and **3**.

| Microorganism | Substrate | Isolated Product | % Yield | Optical Rotation |
|---|---|---|---|---|
| *Aspergillus niger* . . . . . | β-ionone | **3** | 61 | − 5.9° |
| | | **4** | 8 | — |
| | α-ionone[a] | **9** | 40 | + 6.5° |
| *Cunninghamella blakesleeana* . . . | β-ionone | **6** | 28 | −12.3° |
| | | **7** | 23 | — |
| | α-ionone[a] | **9** | 8 | + 6.5° |
| | | **10** | 7 | −34.0° |
| | | **11** | 14 | +70.1° |

[a]Two other compounds were apparent by tlc analysis, but in insufficient quantity to be identified.

analysis of camphanic acid esters of **3** this method was used to assign the enantiomeric composition of the other optically active metabolites of **2** and **8**.

The assignment of the enantiomeric composition at the 9 position for **6** was made using the chemical shift positions of the vinylic protons at C-7. These protons are part of the $L^3$ group as designated by Dale *et al.* (23). The doublet observed was at δ 6.22 for the *S* isomer and at δ 6.32 for the *R*, with an *S*/*R* ratio of 1.75:1. In the case of compound **10** the 5-Me ($L^2$) protons were used to make the determination of configuration at C-3. The *S* isomer showed a singlet at δ 1.62 and the *R* at δ 1.65. In this case, and *S*/*R* ratio of 1.5:1 was observed for this compound.

Interestingly, *C. blakesleeana* seems to preferentially metabolize one isomer of α-ionone to **9**. As expected, the racemic **8** used here does not exhibit an optical rotation, but its metabolite **9** has a significant specific rotation (+6.5°). This information suggests that this fungus selectively metabolizes one of the enantiomers of the racemic substrate.

A recent study of the microbial biotransformation of **2** (18) investigated the possibility that metabolites of **2** might serve as tobacco-flavoring compounds. In this study only **2** was evaluated as a substrate for microbial transformation. Interestingly, these workers also found that a strain of *A. niger* (JTS 191) was active in transforming **2**, although we isolated fewer biotransformation products. We also find that *A. niger* ATCC 16888 readily oxidizes **8** at the allylic 3 position. However, in our experiments we find that *C. blakesleeana* produced metabolites of **2** and **8** most of which were the product of both reduction and oxidation. The methods developed in this model study should prove applicable to the biotransformation of **1** and **12** for use in studies of the identity and stereochemistry of their metabolites.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—$^1$H-nmr spectra were recorded using an IBM AF/250 spectrometer with $CHCl_3$ as an internal standard. Ir spectra were determined with a Beckman 4320 infared spectrophotometer as liquid films. Uv spectra were recorded with a Beckman DU-40 spectrophotometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Electron-impact high resolution mass spectra were obtained with Kratos MS-30 spectrometer. Gc-ms was done utilizing a Kratos MS-25RFA equipped with a Chrompak CPSil 5 column (26 m × 0.32 mm, 2 ml/min) at 130°. Tlc was performed on 0.25-mm Si gel 60 $F_{254}$ precoated glass plates from EM reagents. Cc was done using Si gel as the stationary phase (70–230 mesh, EM Science). Hplc was carried out on a Beckman 332 gradient liquid chromatograph equipped with a Beckman 164 uv detector using a DuPont Zorbax-Sil (4.6 mm × 25 cm) column at a flow rate of 1.5 ml/min. Commercial Ar was dried by bubbling through concentrated $H_2SO_4$ followed by anhydrous $CaSO_4$/KOH. All organic solvents were appropriately dried prior to use. All reagent chemicals were purchased from Aldrich Chemical Co.

SYNTHESIS OF 4-HYDROXY-β-IONONE [**3**].—This compound was prepared by methods that we have previously described. The spectral data obtained also matched that previously reported (19): tlc 40% EtOAc/$C_6H_{14}$ $R_f$ 0.33.

SYNTHESIS OF 4-OXO-β-IONONE [**4**].—This compound was prepared from **3** by methods previously reported. The spectral data obtained was consistent with that found previously (19): tlc 40% EtOAc/$C_6H_{14}$ $R_f$ 0.44.

SYNTHESIS OF 5,6-EPOXY-β-IONONE [**13**].—In 25 ml of ice-cooled dry $CH_2Cl_2$ were dissolved 192 mg (1 mmol) of β-ionone and 238 mg (1.1 mmol) of 80% *m*-chloroperbenzoic acid. The mixture was allowed to warm to room temperature and stirred for 4 h. The solution was then washed twice with cold saturated $NaHCO_3$, then with $H_2O$ followed by saturated NaCl, and dried ($Na_2SO_4$). Filtration and concentration afforded crude **13** which was purified by cc (20% EtOAc/$C_6H_{14}$) to yield 206 mg (ca. 100%) of **13**: tlc [EtOAc-$C_6H_{14}$ (1:5)] $R_f$ 0.33; uv ($CH_2Cl_2$) λ max 234 (ε 14270); ir 2940, 1700, 1680, 1635 $cm^{-1}$; $^1$H nmr ($CDCl_3$) δ 0.94 (s, 3, 5-Me), 1.14 (s, 6, 1-Me), 2.26 (s, 3, H-10), 6.27 (s, 1, H-8, $J$ = 16 Hz), 7.02 (s, 1, H-7, $J$ = 16 Hz); ms *m/z* (rel. int.) $[M - Me]^+$ 193 (4), 177 (38.2), 123 (100).

SYNTHESIS OF RACEMIC 4-CAMPHANOYLOXY-β-IONONE [**5**].—To 20.8 mg (0.1 mmol) of **3** and

22.4 mg (0.22 mmol) of triethylamine in 5 ml of dry THF was added 43.5 mg (0.2 mmol) of *R*-camphanoyl chloride. The reaction mixture was stirred for 18 h at which time the incomplete reaction was diluted with 60% EtOAc/$C_6H_{14}$ and washed with saturated $NaHCO_3$, $H_2O$, and saturated NaCl and dried ($Na_2SO_4$). Filtration and concentration afforded a crude mixture of **5** and **3**. Cc (40% EtOAc/$C_6H_{14}$) afforded pure **5** for analysis: tlc [EtOAc-$C_6H_{14}$ (2:3)] $R_f$ 0.35; $^1$H nmr ($CDCl_3$) δ 0.87–1.25 (m, 12), 1.69 (s, 3, 5-Me), 2.32 (s, 3, H-10), 5.39 (m, 1, CHOAcyl), 6.11 (d, 1, H-8, $J$ = 15 Hz), 7.17 (d, 1, H-7, $J$ = 15 Hz); ms *m/z* (rel. int.) $[M]^+$ 388 (1.8), 373 (16.8), 190 (72.3), 43 (100); hrms *m/z* required for $C_{23}H_{32}O_5$, 388.2251; observed 388.2289.

Hplc analysis (15% EtOAc/$C_6H_{14}$) showed equal amounts of two components eluting with retention times of 21.8 and 22.6 min (*R,S* and *R,R* diastereomers, respectively) (20).

CULTURE METHODS.—*A. niger* and *C. blakesleeana* were obtained from the American Type Culture Collection. These cultures were maintained on Mycophil (Difco) agar slants stored at 4°. Transfer to fresh agar every 2–3 months preserved the cultures.

Shaken cultures for biotransformation experiments were generated by a two-stage fermentation procedure (11) in medium consisting of (per liter of $H_2O$): Pharmamedia (Traders Oil Mill Co., Fort Worth, TX), 10 g; yeast extract, 5 g; D-glucose, 20 g; NaCl, 5 g; $K_2HPO_4$, 5 g. Stage I cultures were initiated by pipetting 1 ml of an aqueous spore suspension prepared from 7-day-old slant cultures into 100 ml of medium in a 500-ml Erlenmeyer flask. The Stage I cultures were incubated on a gyrotory shaker for 2 days (250 rpm, 25°), and then 10 ml of culture was used to inoculate each Stage II culture containing the same medium. One day after initiating Stage II cultures, 20 mg of **2** or **8** in 0.4 ml of EtOH was added for the biotransformation studies.

SCREENING METHODS.—Stage II cultures were incubated with **2** for 7 days, then filtered and the aqueous phase exhaustively extracted with 60% EtOAc/$C_6H_{14}$. Extracts were washed with saturated NaCl, dried ($Na_2SO_4$), and concentrated. Product oils were analyzed by tlc (40% EtOAc/$C_6H_{14}$ or 10% $Me_2CO/CH_2Cl_2$), which was visualized by fluorescence quenching under 254-nm uv light and by charring of plates dipped in 2.5% vanillin in 95% EtOH/$H_2SO_4$. Certain components were tentatively identified by comparison with synthetic standards. Those organisms that exhibited promising results were used for the biotransformation studies of **2** and **8**.

TIME-COURSE METHODS.—The time course of ionone biotransformation was monitored using extracts of the selected cultures which were prepared and analyzed as above. Extracts were prepared 1, 4, and 7 days after addition of **2** and 3, 5, and 7 days after addition of **8**.

PRODUCTION OF BIOTRANSFORMATION PRODUCTS OF **2** AND **8**.—After initiation of cultures as previously described, 20 mg of **2** was added to ten flasks containing *A. niger* for biotransformation. On the seventh day after addition of **2**, the metabolic products were isolated and analyzed by the methods described above. The biotransformation products were purified via cc (40% EtOAc/$C_6H_{14}$). Similar methods were utilized for the biotransformation of **2** (180 mg) by *C. blakesleeana*. However, in this case the products were isolated on days 1, 4, and 7 after the addition of **2**, and a solvent system of 20 to 30% EtOAc/$C_6H_{14}$ was used for chromatography.

The biotransformation of 480 mg of **8** by *A. niger* and of 400 mg of **8** by *C. blakesleeana* was performed using methods similar to those above. The products obtained were purified using a solvent system consisting of 10% $Me_2CO/CH_2Cl_2$.

CHARACTERIZATION OF 4-HYDROXY-β-IONONE [**3**] (*A. NIGER*).—Following purification by cc (40% EtOAc/$C_6H_{14}$), 131 mg (61%) of pure **3** was obtained. This compound possessed spectral and chromatographic properties identical to those of synthetic **3**. The biosynthetic **3** showed an optical rotation of $[\alpha]^{22}D$ −5.9° (*c* = 1.0, MeOH).

*R*-Camphanoylation of 68 mg of microbial **3** for 48 h as above yielded 76 mg (60%) of **5** after cc. Hplc analysis of the resulting **5** diastereomers showed two components with identical retention times as above but with a 6.7:1 *R,R/R,S* ratio.

CHARACTERIZATION OF 4-OXO-β-IONONE [**4**] (*A. NIGER*).—Chromatography (40% EtOAc/$C_6H_{14}$) afforded 18 mg (8%) of pure **4**. This compound had the same spectral and chromatographic properties as its synthetic counterpart.

CHARACTERIZATION OF 4-OXO-β-IONOL [**6**] (*C. BLAKESLEEANA*).—After chromatography (20% EtOAc/$C_6H_{14}$), 55.3 mg (28%) of a yellow oil **6** was isolated: tlc [EtOAc-$C_6H_{14}$ (1:1)] $R_f$ 0.47; uv (MeOH) λ max 264 (ε 10630); ir 3420, 2960, 1670, 1650 $cm^{-1}$; $^1$H nmr δ 1.15 (s, 6, 1-Me), 1.37 (d, 3, 10H, $J_{9,10}$ = 6.5 Hz), 1.81 (s, 3, 5-Me), 1.83 (m, 2, 3H), 2.50 (t, 4, 4H), 4.47 (m, 1, 9H), 5.72 (d × d, 1, 8H, $J_{7,8}$ = 16 Hz, $J_{8,9}$ = 6.5 Hz), 6.23 (d, 1, 7H, $J_{7,8}$ = 16 Hz); ms *m/z* (rel. int.) $[M]^+$ 208 (29.5), 165 (100), 41 (74.5); $[\alpha]^{24}D$ −12.3° (*c* = 1.0, MeOH).

CHARACTERIZATION OF 4-OXO-7,8-DIHYDRO-β-IONONE [7] (*C. BLAKESLEEANA*).—Chromatography (30% EtOAc/$C_6H_{14}$) yielded 44.0 mg (23%) of pure **7**: tlc [EtOAc/$C_6H_{14}$ (1:1)] $R_f$ 0.53; uv (MeOH) λ max 245.5 (ε 14538); ir 2960, 2930, 2875, 1720, 1670 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) 1.15 (s, 6, 1-Me), 1.74 (s, 3, 5-Me), 1.78–1.85 (m, 4, H-2, H-7), 2.19 (s, 3, H-10), 2.43–2.52 (m, 4, H-3, H-8); ms *m/z* (rel. int.) $[M-Me]^+$ 193 (27.5), $[M-OAc]^+$ 165 (100), 137 (25.6).

CHARACTERIZATION OF 3-OXO-α-IONONE [9] (*A. NIGER*).—Pure fractions of **9** (10% acetone/$CH_2Cl_2$) were combined to obtain 207.0 mg (40%) of a yellow oil: tlc (10% $Me_2CO/CH_2Cl_2$) $R_f$ 0.57; uv (MeOH) λ max 237.5 (ε 14012); ir 2960, 2940, 1670, 1665, 1655; $^1H$ nmr ($CDCl_3$) δ (s, 3, Me), 1.04 (s, 3, Me), 1.86 (s, 3, 5-Me), 2.11 (d, 1, 2H, $J_{AB}$ = 17 Hz), 2.25 (s, 3, 9-Me), 2.33 (d, 1, H-2, $J_{AB}$ = 17 Hz), 2.68 (d, 1, H-6, $J_{6,7}$ = 9.5 Hz), 5.95 (s, 1, H-4), 6.15 (d, 1, H-8, $J_{7,8}$ = 16 Hz), 6.62 (d × d, 1, H-7, $J_{6,7}$ = 9.5 Hz, $J_{7,8}$ = 16 Hz); ms *m/z* (rel. int.) $[M]^+$ 206 (1.29), 150 (21.8), 108 (100), 43 (54.3); $[\alpha]^{22}D$ +6.5° (*c* = 0.6, THF).

CHARACTERIZATION OF 3-OXO-α-IONONE [9] (*C. BLAKESLEEANA*).—Pure **9** (10% $Me_2CO/CH_2Cl_2$), 35.5 mg (8%), was obtained as a yellow oil. This material exhibited the same spectral and chromatographic properties as that isolated from the *A. niger* incubations.

CHARACTERIZATION OF 3-HYDROXY-α-IONONE [10] (*C. BLAKESLEEANA*).—The fractions (10% $Me_2CO/CH_2Cl_2$) containing **10** were combined to afford 31.3 mg (7%): tlc (10% $Me_2CO/CH_2Cl_2$) $R_f$ 0.32; uv (MeOH) λ max 225 (ε 12000); ir 3410, 2960, 2920, 2870, 1675, 1620 $cm^{-1}$; $^1H$ nmr 0.86 (s, 3, 1-Me), 1.00 (s, 3, 1-Me), 1.38 (d × d, 1, H-2, $J_{AB}$ = 3.5 Hz, $J_{2,3}$ = 6.4 Hz), 1.59 (s, 3, 5-Me); 1.81 (d × d, 1, H-2, $J_{AB}$ = 13.5 Hz, $J_{2,3}$ = 6.0 Hz), 2.23 (s, 3, 9-Me), 2.47 (d, 1, H-6, $J_{6,7}$ = 10 Hz), 4.24 (m, 1, OH), 5.60 (m, 1, H-3), 6.07 (d, 1, H-8, $J_{7,8}$ = 16 Hz), 6.49 (d × d, 1, H-7, $J_{6,7}$ = 16 Hz, $J_{7,8}$ = 10 Hz); ms *m/z* (rel. int.) $[M]^+$ 208 (3.98), 109 (95.4), 43 (100); $[\alpha]^{22}D$ −34.0° (*c* = 1.0, MeOH).

CHARACTERIZATION OF 3-HYDROXY-α-IONOL [11] (*C. BLAKESLEEANA*).—Compound **11** was isolated as a relatively pure material, 61.3 mg (14%) (10% $Me_2CO/CH_2Cl_2$). However, **11** slowly oxidizes to **10** upon isolation. Due to this oxidation of **11** the reported spectral data may be regarded as tentative: tlc (10% $Me_2CO/CH_2Cl_2$) $R_f$ 0.14; uv (hexane) 223.5 (ε 807); ir 3390, 2970, 2930, 2880, 1640 $cm^{-1}$; $^1H$ nmr 1.88 (d, 1, H-10, $J_{9,10}$ = 1.5 Hz), 2.49 (d, 1, H-6, $J_{6,7}$ = 8.4 Hz), 4.31 (m, 1, H-9), 5.65 (d × d, 1, H-7, $J_{6,7}$ = 8.4 Hz, $J_{7,8}$ = 16 Hz), 5.86 (m, 1, H-4); ms *m/z* (rel. int.) 192 (18.3), 174 (7.3), 108 (100); $[\alpha]^{22}D$ +70.1° (*c* = 1.0, $CH_2Cl_2$).

SYNTHESIS OF *S*-(+)-α-METHOXY-α-TRIFLUOROMETHYLPHENYLACETYL CHLORIDE (21).—*R*-(+)-α-Methoxy-α-trifluoromethylphenylacetic acid (1 g, 4.3 mmol), thionyl chloride (1.84 ml, 25 mmol), and NaCl (12.4 mg, 0.2 mmol) were refluxed for 51 h. The reaction mixture was allowed to cool to room temperature, and volatile components were removed under reduced pressure. Azeotropic distillation with five 15-ml portions of $C_6H_6$ removed the last traces of thionyl chloride. The resulting residue was subjected to a 1.5-mm atmosphere for 1 h to give 727 mg (67%) of a yellow oil.

SYNTHESIS OF THE *R*-(+)-MPTA DERIVATIVES.—The derivatives of **3**, **6**, and **10** were prepared according to the method outlined by Dale *et al.* (23). Excess MPTACl was hydrolyzed using 20 μl of saturated $NaHCO_3$. The reaction was diluted with $Et_2O$ and successively washed with cold 3 N HCl, cold saturated $NaHCO_3$ and brine and dried ($Na_2SO_4$). After filtration to remove the $Na_2SO_4$, the solution was concentrated at reduced pressure to yield a yellow oil in each case.

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