Chemical and microbiological remote functionalisation of (+)- and (−)-bornyl acetate[1]

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Chemical oxidation of (−)-bornyl acetate provides a mixture of 3-, 5-, and 6-oxobornyl acetate, whereas microbiological hydroxylation with cultures of *Helminthosporium sativum* gives a mixture of 2,3-, 2,6-, and 2,5-bornanediols. In each case the reaction occurs preferentially at the C(5) position. Microbiological hydroxylation of (+)-bornyl acetate with *H. sativum* occurs almost exclusively at the C(5) position. Regiospecific hydroxylation of (+)- or (−)-bornyl acetate with cultures of *Fusarium culmorum* also occurs at the C(5) position but without concomitant hydrolysis of the acetoxy group.

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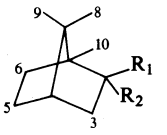
L'oxydation chimique de l'acétate du (−)-bornyle conduit à un mélange des acétates des oxo-3, -5 et -6 bornyles; l'hydroxylation microbiologique par des cultures d'*Helminthosporium sativum* fournit un mélange des bornane-diols-2,3 -2,6 et -2,5. Dans chaque cas, la réaction se produit préférentiellement en position C(5). L'hydroxylation microbiologique de l'acétate du (+)-bornyle par le *H. sativum* se produit presqu'exclusivement en position C(5). L'hydroxylation régiospécifique des acétates des (+)- ou (−)-bornyles par des cultures de *Fusarium culmorum* se produit aussi en position C(5); elle n'est toutefois pas accompagnée d'une hydrolyse du groupe acétoxy.

[Traduit par le journal]

As part of our synthetic studies (2 and references cited therein) in the monoterpenoid and sesquiterpenoid area we have considered the possibility of using remote oxidation or hydroxylation reactions to convert commercially available or synthetically accessible mono- and sesquiterpenoids to more complex, oxygenated derivatives. Our interest in this approach to terpenoid synthesis was prompted by a consideration of biosynthetic evidence which demonstrates that the introduction of hydroxyl or carbonyl groups into unactivated positions (i.e., remote from activating functionality) is a characteristic feature of many biosynthetic sequences. In addition there are many reports in the literature which indicate that transformations of this type can be accomplished in the laboratory using microbiological (3) or chemical techniques (4). Since our immediate synthetic objectives were dependent on the synthesis of 2,5-disubstituted bornane (camphane) derivatives we directed our initial attention to the direct remote oxidation or hydroxylation of bornyl (**1**) and isobornyl acetate (**2**).[2]



1 $R_1 = H, R_2 = OAc$
2 $R_1 = OAc, R_2 = H$

Remote Oxidation of (−)-Bornyl Acetate (1) with CrO_3/HOAc or CrO_3/HOAc/Ac_2O

Several reports in the literature have established that oxidation of bornyl acetate with CrO_3/HOAc (6) or CrO_3/Ac_2O/HOAc (7) provides 5-oxobornyl acetate (**3**) and its 6-oxo isomer (**8**) in ~15–35% yield. In our initial investigations we reexamined this remarkable transformation to assess its potential use in synthetic and biosynthetic studies.

Oxidation of (−)-bornyl acetate (**1**) with CrO_3/Ac_2O/HOAc (7) for 7 days at 0–25°C provided a mixture of products which was partially purified and separated into components by crystallisation, column chromatography, and/or preparative glc. The major product (~40% yield, see Table 1), readily isolated by crystallisation, was identified as 5-oxobornyl acetate (**3**) (7*c*) on the basis of its spectroscopic properties and conversion to 5-oxoborneol (**4**), 5-*exo*-hydroxyborneol (**5**) (7*c*), and bornan-2,5-dione (**6**) (7*c*). Minor components of the reaction mixture were identified as 5-*exo*-acetoxybornyl acetate (**7**), 6-

[1]The results described in this paper were previously reported in preliminary communications (1).

[2]Remote oxidation of isobornyl acetate (**2**) (5; M. S. Allen, D. H. Hunter, and T. Money. Unpublished observations) and its use in the synthesis of nojigiku alcohol and monoterpenoid analogs of *cis*-sativenediol, *trans*-sativenediol, and helminthosporal will be described in later papers (17).

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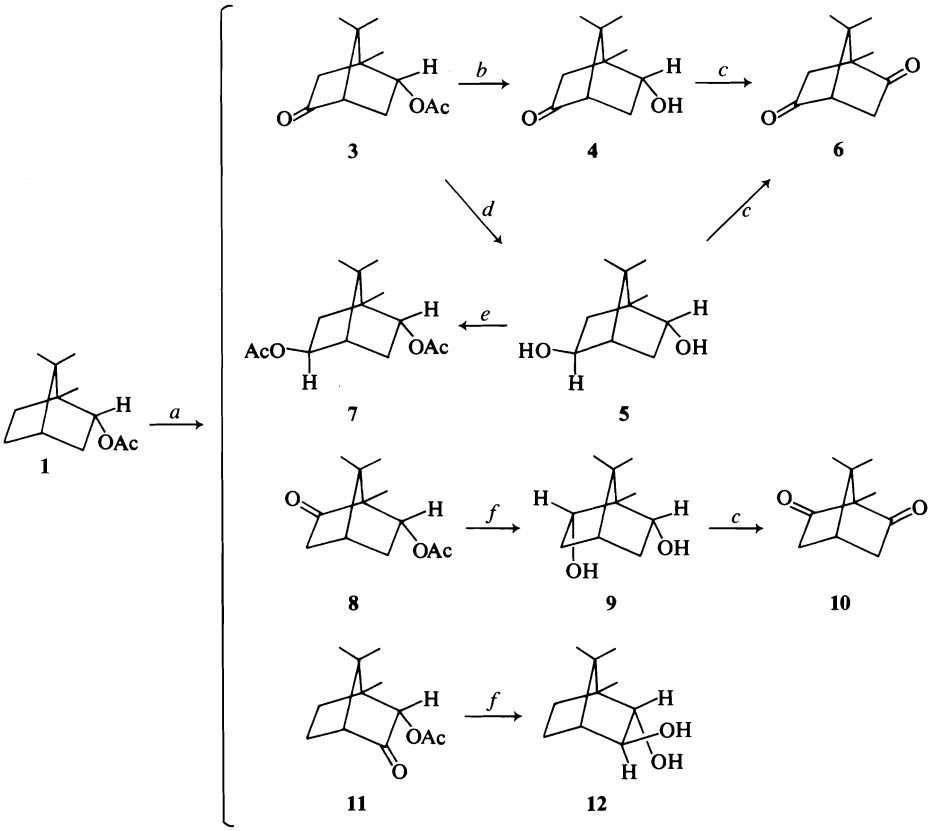
TABLE 1. Chemical oxidation of (−)-bornyl acetate (**1**)

Product	Yield (%)[a]	
	CrO_3/HOAc/Ac_2O	CrO_3/HOAc
(−)-Bornyl acetate (**1**)	2	5
Camphor	—	8
5-Oxobornyl acetate (**3**)	40	24
6-Oxobornyl acetate (**8**)	16	5
3-Oxobornyl acetate (**11**)	2	2
5-*exo*-Acetoxybornyl acetate (**7**)	6	1
Bornane-2,5-dione (**6**)	1	2
Bornane-2,6-dione (**10**)	5	—

[a]Estimated by glc analysis (prep. 30% SE-30 and QF-1) of total reaction product using samples of individual components as standards.



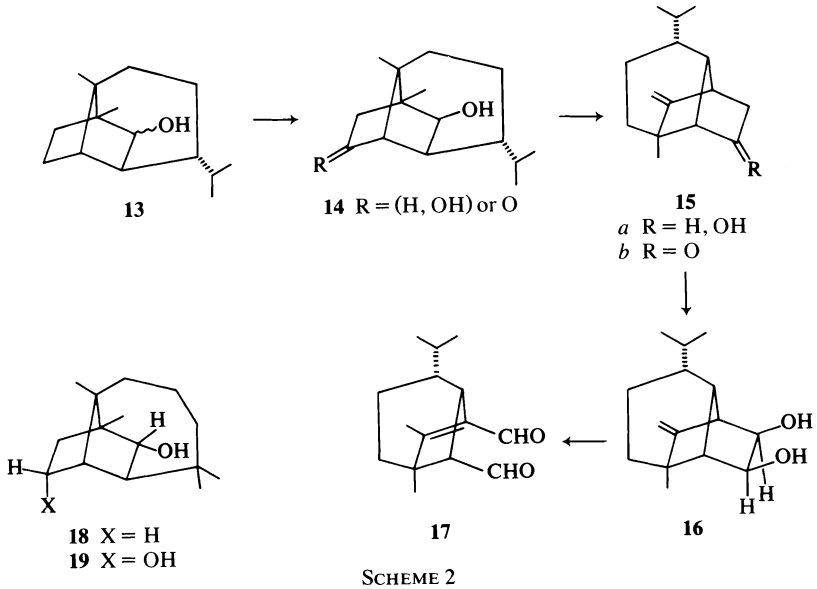
SCHEME 1. (*a*) CrO_3/Ac_2O/HOAc; (*b*) Na_2CO_3/H_2O/MeOH; (*c*) $C_5H_5N^+H\cdot CrO_3\cdot Cl^-$; (*d*) $LiAlH(OMe)_3$; (*e*) Ac_2O/C_5H_5N; (*f*) $LiAlH_4$.

oxobornyl acetate (**8**), and 3-oxobornyl acetate (**11**) by chemical correlation with bornan-2,6-dione (**10**),[3] 6-*endo*-hydroxyborneol (**9**), bornan-2,5-dione (**6**),[3] and 3-*exo*-hydroxyborneol (**12**) (8) (Scheme 1). The stereochemistry of the acetoxy group in **7** was established by its nmr spectrum and its synthesis from 5-*exo*-hydroxyborneol (**5**). Oxidation of (−)-bornyl acetate with CrO_3/HOAc (6) produced a similar mixture of products in lower yield (Table 1).

Microbiological Hydroxylation of (−)- and (+)-Bornyl Acetate

The regiospecificity of the oxidative transformation described above led us to consider that a similar functionalisation of the C(5) position in more complex camphane systems could occur during the biosynthesis of several groups of sesquiterpenoids. For

[3]Bornane-2,5-dione (6) and bornane-2,6-dione (10 and references cited therein) were also identified as minor components of the oxidation process.



13 **14** R = (H, OH) or O **15** *a* R = H, OH *b* R = O **16** **17** **18** X = H **19** X = OH

SCHEME 2

TABLE 2. Hydroxylation of (−)-bornyl acetate (**5**) by *H. sativum*[4]

Product	Yield (%)[a]		
(−)-5-*exo*-Hydroxyborneol (**5**)	17	24	29
(−)-5-*endo*-Hydroxyborneol (**20**)	6	10	8
(−)-3-*exo*-Hydroxyborneol (**12**)	11	12	15
(−)-6-*exo*-Hydroxyborneol (**21**)	5	6	8

[a]For analytical procedure see Experimental.

example introduction of oxygen functionality into an ylangobornane derivative (**13**; OH *endo* or *exo*), followed by Wagner–Meerwein rearrangement of **14** to a 5-oxo- (**15***a*) or 5-hydroxysativene (**15***b*) could be involved in the biosynthesis of *cis*-sativenediol (**16**), helminthosporal (**17**), and related compounds produced by *Helminthosporium sativum* (cf. ref. 9) (Scheme 2).[4] Similarly we considered that the final step (?) in the biosynthesis of culmorin (**19**) (cf. ref. 10), a metabolite of *Fusarium culmorum*, could involve C(5)-hydroxylation of longiborneol (**18**) or a suitable derivative. A result of these biosynthetic considerations is the prediction that *H. sativum* and *F. culmorum* contain a C(5)-hydroxylase which may be capable of introducing oxygen functionality at the C(5) position in compounds such as borneol[5] or more complex compounds such as longiborneol, etc. Support for these predictions is described below.

Hydroxylation of (−)- and (+)-Bornyl Acetate with H. sativum[4]

(−)-Bornyl acetate (**1**) was added to 3-day-old cultures of *H. sativum* and, after 7–10 days, ether extraction of the broth provided (−)-borneol and a mixture of bornanediols which were separated by column chromatography. On the basis of their nmr spectra and chemical correlation with products obtained in the chemical oxidation of **1**, the various microbiological products were identified as (−)-5-*exo*-hydroxyborneol (**5**) (major), (−)-5-*endo*-hydroxyborneol (**20**), (−)-6-*exo*-hydroxyborneol (**21**), and (−)-3-*exo*-hydroxyborneol (**12**) (8). The overall yield of diols was ~50% and the relative proportions of 2,5-, 2,3-, and 2,6- isomers (~5:2:1) was calculated from glc analysis of their diacetates (Table 2).[6,7]

When (+)-bornyl acetate (**22**) was used as substrate with *H. sativum* the regiospecificity of hydroxylation increased considerably and the only major products were (+)-5-*exo*- (**23**) and (+)-*endo*-hydroxylborneol (**24**).[8] The yield of diols **23** and **24** was ~35–65% and their relative proportion, as

[4]Now classified as *Bipolaris sorokiniana*.

[5]Previous studies (3*a*, *b*) have shown that the methylene groups of camphor, fenchone, and isofenchone can be hydroxylated by microorganisms or animals.

[6]Other minor components of the reaction mixture have been tentatively identified as 6-ketoborneol and bornane-2,8-diol.

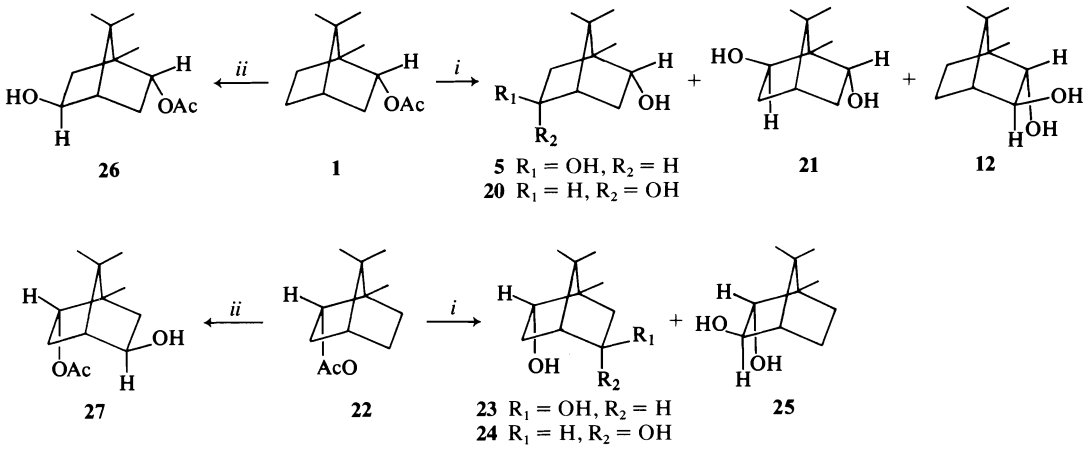
[7]We are unable to provide a satisfactory explanation for the variation in relative yields of products in successive microbiological experiments (see Tables 2 and 3).

[8]The only other reaction product, (+)-3-*exo*-hydroxyborneol (**25**) was formed in ~2% yield (see Table 3).

TABLE 3. Hydroxylation of (+)-bornyl acetate (**22**) by *H. sativum*

Product				
(+)-5-*exo*-Hydroxyborneol (**23**)	56	32	47	38
(+)-5-*endo*-Hydroxyborneol (**24**)	8	4	11	16
(+)-3-*exo*-Hydroxyborneol (**25**)	3.5	1	2	2

[a]For analytical procedure see Experimental.



(*i*) *Helminthosporium sativum*; (*ii*) *Fusarium culmorum*.

SCHEME 3

determined by glc and nmr of the corresponding diacetates, varied from ~2.5:1 to ~7:1 (Table 3).[7]

Hydroxylation of (−)- and (+)-Bornyl Acetate with F. culmorum

Addition of (−)-bornyl acetate (**1**) to 7-day-old cultures of *F. culmorum* and work-up after 18 days provided 5-*exo*-hydroxybornyl acetate (**26**) as the only major product (~12% yield). The identity of this product was established by its spectroscopic properties and by its conversion to (−)-5-*exo*-acetoxybornyl acetate (**7**) and 5-oxobornyl acetate (**3**). An almost identical result was obtained when (+)-bornyl acetate (**22**) was used as substrate (Scheme 3).

The efficiency, regiospecificity, and stereoselectivity of the microbiological hydroxylations described above compare favourably with microbial transformations of other terpenoids (3*a–e*). In addition the regiospecificity of hydroxylation of (+)-bornyl acetate by *H. sativum* and the ability of *F. culmorum* to hydroxylate at C(5) while leaving the C(2)-acetoxy group intact[9] could be of value in subsequent synthetic studies.

[9]Preliminary observations (R. Zerr. Unpublished results) indicate that *F. culmorum* converts isobornyl acetate (2) to 5-hydroxyisobornyl acetate. Hence 5-oxo-isobornyl acetate is accessible by a microbiological route which complements the laboratory synthesis involving direct oxidation with CrO_3/HOAc/Ac_2O (5; M. S. Allen, D. H. Hunter, and T. Money. Unpublished results).

The remarkable correspondence in regiospecificity between the chemical and microbiological reactions described above probably reflects the greater accessibility of the C(5) position towards oxidising agents or enzymic systems. We have suggested a similar explanation for the partial regiospecificity of other direct remote oxidation reactions (11).

Experimental

Chromium trioxide (Mallinckrodt, Technical grade 99.75%), (−)-bornyl acetate (Aldrich Chemical Company), and (+)-camphor (Eastman Organic Chemicals) were used as received. (+)-Bornyl acetate, $[\alpha]_D$ +43.2° (*c* 2.47, $CHCl_3$), was prepared by calcium – liquid ammonia reduction (12) of (+)-camphor followed by column chromatography (alumina) and acetylation. Pyridine was dried over potassium hydroxide pellets before use and methylene chloride for pyridinium chlorochromate oxidations was dried by distillation from phosphorus pentoxide.

Nuclear magnetic resonance spectra were recorded at 100 MHz using either a Varian HA-100 or XL-100 machine, or the HA-100 magnet with Bruker TT23 console and Nicolet 16 K computer. Infrared spectra were recorded with Perkin Elmer 137 or 710A instruments.

Column chromatography was performed with aluminium oxide Woelm neutral, for column chromatography (ICN Pharmaceuticals) or with silicic acid, 100 mesh (Mallinckrodt). Alumina was deactivated to grade III before use. Thin-layer chromatography plates were made from silica gel GF-254 for tlc (E. Merck) and were visualised by spraying with a saturated solution of ceric sulphate in 12 *N* aqueous sulphuric acid followed by heating.

Gas–liquid chromatography was performed with either a Varian Aerograph model 90-P or Hewlett-Packard HP5831A

instrument, using thermal conductivity and flame ionisation detectors respectively. Analytical glc on the Varian instrument was conducted with 6 ft × 0.25 in. stainless steel columns using 3% SE-30, 10% OV-210, or 20% DEGS stationary phases on 60–80 or 80–100 mesh Chromosorb W. Preparative glc was performed with a 10 ft × 0.375 in. stainless steel column with 20% DEGS as the stationary phase on 60–80 mesh Chromosorb W. Mass spectra were recorded at 70 eV with a Varian/MAT CH4B spectrometer and high resolution mass measurements were determined with a Kratos-AEI MS50 instrument. Gas–liquid chromatography on the Hewlett-Packard instrument was performed with 6 ft × 0.125 in. stainless steel columns, using 3% OV-17, and 10% Carbowax stationary phases on 80–100 mesh Chromosorb W-HP support.

Optical rotations were measured with a Perkin-Elmer 141 or 241MC instrument and microanalyses were performed by Mr. P. Borda, Microanalytical Laboratory, University of British Columbia, Vancouver.

Oxidation of (−)-Bornyl Acetate with Chromium Trioxide in Acetic Anhydride – Acetic Acid

A solution of (−)-bornyl acetate (**1**) (60 g, 0.31 mol) in glacial acetic acid (260 mL) and acetic anhydride (115 mL) was cooled to 0°C. The solution was stirred and maintained at 0°C while a solution of chromium trioxide (85 g, 0.85 mol, 2.1 equiv.) in acetic anhydride (135 mL) was added in several portions over 24 h. After this time the mixture was allowed to warm to room temperature and stirring was continued at this temperature for a further 6 days. The green viscous reaction mixture was dissolved in water (2500 mL), extracted with ether (3 × 650 mL), using filtration through Celite 535 to aid phase separation, and the ether extracts washed successively with water, excess aqueous sodium bicarbonate, and saturated aqueous sodium chloride. Drying (Na_2SO_4) and evaporation provided a viscous yellow oil. Separation of the oxidation products (27.2 g; bp 90–96°C/0.85 Torr) from recovered starting material was achieved by fractional distillation *in vacuo*.

Typical Oxidation Procedure using Chromium Trioxide in Glacial Acetic Acid

A solution of (−)-bornyl acetate (**1**) (20 g, 0.10 mol) in glacial acetic acid (30 mL) was stirred and heated to reflux while a slurry of chromium trioxide (50 g, 0.50 mol, 3.75 equiv.) in glacial acetic acid (70 mL) was cautiously added in small portions over 40–80 min. A further portion of glacial acetic acid (70 mL) was used to wash the oxidising agent into the reaction vessel. Heating and stirring were continued for 30–80 min and the solution was cooled, diluted with water (600 mL), and extracted with ether (3 × 350 mL). The ether extracts were washed successively with excess aqueous sodium bicarbonate and saturated aqueous sodium chloride, dried (Na_2SO_4), and evaporated to give a pale yellow viscous oil. Separation of the oxidation products (7.3 g; bp 89–90°C/0.8 Torr) from unreacted starting material was achieved by fractional distillation *in vacuo*.

*Isolation of (−)-5-Oxobornyl Acetate (**3**)*

The fractionally distilled product from the oxidation of (−)-bornyl acetate with chromium trioxide in acetic acid readily crystallised upon standing at −20°C. Washing with ice-cold hexane followed by crystallisation from hexane gave (−)-*5-oxobornyl acetate* (**3**) as a colourless solid, mp 78°C (lit. (7*b*) mp 78°C, lit. (7*c*) mp 74–76°C); $[\alpha]_D^{25}$ −102.6° (*c* 1.96, $CHCl_3$) (lit. (7*c*) $[\alpha]_D$ −97° in $CHCl_3$)); ν_{max} (CCl_4): 1745, 1235, 1035 cm^{-1}; δ ($CDCl_3$): 0.94, 1.00, 1.01 (3H each, s, CH_3), 1.30 (1H, d of d, *J* = 3.5 and 14 Hz, 3-*endo*-H, collapses to d, *J* = 14 Hz, upon irradiation at 5.06 δ), 2.03 (3H, s, CH_3CO_2—), 2.16 (1H, d, *J* = 5 Hz, C(4)-H), 2.35–2.75 (2H, overlapping of d, *J* = 18 Hz (6 *endo*-H) and multiplet (3-*exo*-H), collapsing to d of d, *J* = 14 and 5 Hz, upon irradiation at 5.06 δ), 5.06 (1H, multiplet, collapsing to dd, *J* ~ 9 and 1–2 Hz, upon irradiation at 1.30 δ, 2-*exo*-H); *m/e* (relative intensity): 210(M^+, 24), 168(84), 124(36), 111(23), 109(32), 108(40), 107(34), 43(100), 41(24).

*(−)-5-Oxoborneol (**4**)*

A mixture of (−)-5-oxobornyl acetate (**3**) (1.00 g) and sodium carbonate decahydrate (1.37 g) in methanol (4 mL) and water (7 mL) was briefly heated to reflux and then allowed to stand for 48 h. The mixture was diluted with water, extracted with ether several times, and the ether extracts washed with water, dried (Na_2SO_4), and evaporated. Recrystallisation from cyclohexane gave (−)-*5-oxoborneol* (**4**) as a colourless solid (0.65 g, 81%), mp 239–241°C (sealed capillary) (lit. (7*c*) mp 247–248°C); $[\alpha]_D^{25}$ −87.0° (*c* 1.55, $CHCl_3$) (lit. (7*c*) $[\alpha]_D$ −74° in $CHCl_3$); ν_{max} ($CHCl_3$): 3630, 3480, 1745, 1035 cm^{-1}; δ ($CDCl_3$): 0.91, 0.95, 1.00 (3H each, s, CH_3), 1.27 (1H, d of d, *J* = 14 and 3.5 Hz, 3-*endo*-H), 1.86 (1H, d with fine coupling, *J* = 18 Hz, 6-*exo*-H), 2.13 (1H, d, *J* = 5 Hz, 4-H), 2.3–2.75 (2H, overlapping of doublet, *J* = 18 Hz, 6-*endo*-H, and of multiplet, 3-*exo*-H), 4.20 (1H, multiplet, 2-*exo*-H): d of d at 1.27 δ collapses to d (*J* = 14 Hz) upon irradiation of 4.20 δ; d with fine coupling at 1.86 δ collapses to d (*J* = 18 Hz) upon irradiation at 4.20 δ; *m/e* (relative intensity): 168(M^+, 49), 125(49), 124(100), 109(46), 71(30), 70(39), 55(32), 41(44).

*(−)-5-*exo-*Hydroxyborneol (**5**)*

To a freshly prepared solution of lithium trimethoxy aluminum hydride (13) (11.2 mmol) in tetrahydrofuran (25 mL), stirred under nitrogen at 0°C, was added a solution of 5-oxobornyl acetate (**3**) (250 mg, 1.2 mmol) in tetrahydrofuran (5 mL). Stirring was continued for 30 min at 0°C, then for 17 h at room temperature. Ether (25 mL) was added, followed by just sufficient water to decompose the excess reducing agent and produce a white, easily filtered precipitate. Filtration of the product followed by evaporation of the filtrate and crystallisation from benzene provided (−)-5-*exo*-hydroxyborneol (**5**) as a colourless solid (169 mg, 84%), mp 258–259°C (sealed capillary); $[\alpha]_D^{27}$ −16.3° (*c* 0.81, $CHCl_3$); ν_{max} ($CHCl_3$): 3650, 1040 cm^{-1}; δ ($CDCl_3$): 0.76, 0.81, 1.04 (3H each, s, CH_3), 1.25–1.50 (1H, multiplet), 1.73 (1H, d, *J* = 5 Hz, 4-H), 2.1–2.5 (2H, multiplet), 3.83 (2H, multiplet, 2-*exo*-H and 5-*endo*-H); *m/e* (relative intensity): 170(M^+, 4) 137(18), 126(29), 125(47), 111(100), 109(59), 83(17), 55(18), 43(22), 41(26). *Anal.* calcd. for $C_{10}H_{18}O_2$: C 70.55, H 10.66; found: C 70.50, H 10.76.

*(−)-5-*exo-*Acetoxybornyl Acetate (**7**)*

A solution of (−)-5-*exo*-hydroxyborneol (**6**) (50 mg) in pyridine (1.6 mL, dried by standing over potassium hydroxide pellets) and acetic anhydride (0.4 mL) was heated at 90°C with stirring while under nitrogen for 19 h. The mixture was diluted with water, extracted three times with ether, and the ether extracts washed successively with 1 *N* aqueous hydrochloric acid (excess), water, excess aqueous sodium bicarbonate, and saturated aqueous sodium chloride. The ether extracts were then dried (Na_2SO_4) and evaporated to give (−)-5-*exo*-acetoxybornyl acetate (**7**) as an almost colourless viscous oil (72 mg, 97%). Purification by preparative glc (30% SE-30) gave a colourless viscous oil; $[\alpha]_D^{27}$ −13.6° (*c* 1.49, $CHCl_3$); ν_{max} (CCl_4): 1740, 1230, 1030 cm^{-1}; δ ($CDCl_3$): 0.85, 0.90, 1.01 (3H each, s, CH_3), 1.49 (1H, multiplet), 1.88 (1H, d, *J* = 5 Hz, 4-H), 1.97 and 2.00 (3H each, s, CH_3CO_2—), 2.2–2.6 (2H, multiplet), 4.63 (1H, d of d, 5-*endo*-H), 4.78 (1H, multiplet, 2-*exo*-H); *m/e* (relative intensity): 254(M^+, 25), 195(30), 168(24), 152(27), 137(27), 135(27), 134(70), 126(30), 119(57),

109(73), 108(63), 93(37), 43(100). *Anal.* calcd. for $C_{14}H_{22}O_4$: C 66.12, H 8.72; found: C 66.16, H 8.67.

*Isolation of 6-Oxobornyl Acetate (**8**), 3-Oxobornyl Acetate (**11**), and 5-*exo*-Acetoxybornyl Acetate (**7**)*

The fractionally distilled oxidation product from the oxidation of bornyl acetate with chromium trioxide in acetic acid and acetic anhydride was cooled to −20°C for 7 days to promote crystallisation of 5-oxobornyl acetate (**3**). This was removed by filtration and the mother liquors subjected to column chromatography on silica gel (2.0 g of substrate, 200 g of silica gel deactivated to grade III; eluted with 15% ether in hexane). Two fractions were obtained, each of which was a mixture of two main components by glc (20% DEGS). Each component was isolated by preparative glc. Preparative glc (30% SE-30) of the more rapidly eluted fraction (0.42 g) provided 3-oxobornyl acetate (**11**) and 5-*exo*-acetoxybornyl acetate (**7**) in order of increasing retention time.

3-Oxobornyl acetate (**11**) was obtained as a colourless partially crystalline solid, a single component on tlc (silica gel, ether – petroleum ether 1:2) and glc (3% SE-30 and 5% QF-1); $[\alpha]_D^{25}$ −42.50° (*c* 1.0, $CHCl_3$); ν_{max} (CCl_4): 1755, 1250, 1060 cm^{-1}; δ ($CDCl_3$): 0.98 and 1.01 (6H and 3H respectively, s, CH_3), 2.11 (3H, s, CH_3CO_2—), 2.29 (1H, d, J = 5 Hz, 4-H) 5.19 (1 H, fine coupling, 2-*exo*-H); *m/e* (relative intensity): 210(M^+, 23), 168(39), 123(33), 122(18), 113(14), 81(14), 71(18), 69(12), 58(10), 55(13), 43(100); *Mol. Wt.* calcd. for $C_{12}H_{18}O_3$: 210.1256; found (mass spectrometry): 210.1237.

5-exo-*Acetoxybornyl acetate* (**7**) was obtained as a colourless viscous oil, identical with material from the acetylation of 5-*exo*-hydroxyborneol (**5**) by tlc (silica gel, ether – petroleum ether 1:1) and glc (3% SE-30 and 20% DEGS), as well as by nmr ($CDCl_3$) and ir (CCl_4) spectrometry.

Preparative glc (30% QF-1) of the less rapidly eluted fraction (1.31 g) provided 6-oxobornyl acetate (**8**) and 5-oxobornyl acetate (**3**) in order of increasing retention time.

6-Oxobornyl acetate (**8**) was isolated as a colourless partially crystalline solid, providing colourless crystals from hexane, mp 62–62.5°C; $[\alpha]_D^{25}$ +31.42° (*c* 1.13, $CHCl_3$); ν_{max} (CCl_4): 1750, 1245, 1075, 1050 cm^{-1}; δ ($CDCl_3$): 0.85, 0.94, 1.04 (3H each, s, CH_3), 1.27 (1H, d of d, J = 14 and 3 Hz, collapsing to d, J = 14 Hz, upon irradiation at 5.13 δ, 3-*endo*-H), 1.99 (3H, s, CH_3CO_2—), 5.13 (1H, d of d, J = 9 and 3 Hz, 2-*exo*-H); *m/e* (relative intensity): 210(M^+, 5), 168(46), 167(27), 153(100), 150(19), 121(21), 109(19), 108(58), 107(29), 43(39), 41(18). *Anal.* calcd. for $C_{12}H_{18}O_3$: C 68.55, H 8.63; found: C 68.49, H 8.55.

5-Oxobornyl acetate (**3**) was isolated as a colourless crystalline solid, identical with material previously isolated from the chromium trioxide – acetic acid oxidation of bornyl acetate by tlc (silica gel, ether – petroleum ether 1:2), glc (3% SE-30 and 5% QF-1), as well as by nmr ($CDCl_3$) and ir (CCl_4) spectrometry.

6-endo-*Hydroxyborneol* (**9**)

To a solution of 6-oxobornyl acetate (**8**) (81 mg) in dry ether (2 mL), stirred under nitrogen at −78°C, was added lithium aluminum hydride (37 mg, 3.4 equiv.) in three portions over 15 min. After stirring at −78°C for a further 3 h the mixture was allowed to warm to room temperature and the stirring continued for a further 19 h. Just sufficient water was added to decompose the aluminum salts to a readily filtered precipitate and the mixture was filtered and the filtrate evaporated. This provided a colourless viscous oil which was purified by column chromatography on silica gel (9 g, deactivated to grade III, and eluted with ether – petroleum ether 1:2). This gave 6-*endo*-hydroxyborneol (**9**) (53 mg, 81%), mp 258–260°C (from hexane) (sealed capillary); $[\alpha]_D^{25}$ −0.05 ± 0.06° (*c* 3.46, $CHCl_3$); ν_{max} (1% in $CHCl_3$): 3620, 3480, 1130, 1060 cm^{-1}; δ ($CDCl_3$): 0.84 (6H, s, C(8)- and C(9)-CH_3), 1.06 (3H, s, C(10)-CH_3), 1.34 (2H, d of d, J = 12.5 and 2.5 Hz; collapsing to d, J = 12.5 Hz, upon irradiation at 4.25 δ; collapsing to d, J = 2–3 Hz, upon irradiation at 2.47 δ; 3-*endo*- and 5-*endo*-H), 1.75 (1H, t, J = 5 Hz, collapsing to s upon irradiation at 2.47 δ, C(4)-H), 2.47 (2H, multiplet (d of d of d), J = 12.5, 9.5, and 5 Hz; collapsing to d of d, J = 12.5 and 5 Hz upon irradiation at 4.25 δ; collapsing to d of d, J = 12.5 and 9.5 Hz, upon irradiation at 1.75 δ; collapsing to d of d, J = 9.5 and 5 Hz upon irradiation at 1.34 δ; 3-*exo*- and 5-*exo*-H), 4.25 (2H, d, of d, J = 9.5 and 2.5 Hz, collapsing to doublet, J = 9.5 Hz, upon irradiation at 1.34 δ; collapsing to broad singlet upon irradiation at 2.47 δ; 2-*exo*- and 6-*exo*-H); δ (C_6D_6): 0.64 (6H, s, C(8)- and C(9)-CH_3), 1.08 (3H, s, C(10)-CH_3), 1.25–1.6 (3H, complex, 3-*endo*-, 5-*endo*-, and C(4)-H), 2.33 (2H, m, 3-*exo*- and 5-*exo*-H), 4.19 (2H, m, 2-*exo*- and 6-*exo*-H); δ (CCl_4): 0.82 (6H, s, C(8)- and C(9)-CH_3), 0.98 (3H, s, C(10)-CH_3), 1.30 (2H, poorly resolved d of d, J = 2–3 and 13 Hz, 3-*endo*- and 5-*endo*-H), 1.68 (1H, t, J = 4.5 Hz, C(4)-H), 2.39 (2H, m, 3-*exo*- and 5-*exo*-H), 4.12 (2H, poorly resolved d of d, J = 2–3 and 10 Hz, 2-*exo*- and 6-*exo*-H); *m/e* (relative intensity): 109(17), 108(100), 95(9), 93(18), 68(5), 55(5), 43(6), 41(112), no molecular ion visible. *Anal.* calcd. for $C_{10}H_{18}O_2$: C 70.55, H 10.66; found: C 70.56, H 10.74.

(−)-3-exo-*Hydroxyborneol* (**12**)

To a solution of 3-oxobornyl acetate (**11**) (approximately 10 mg) in dry ether (1 mL), stirred at −78°C under nitrogen, was added lithium aluminum hydride (22 mg). Stirring was continued at −78°C for 4 h then at 25°C for 18 h, and the mixture was worked up by adding just sufficient water to decompose the aluminum salts to a readily filterable precipitate. Filtration and evaporation of the ether followed by column chromatography (silica gel, 0.5 g, elution with ether – petroleum ether 1:2) provided (−)-3-*exo*-hydroxyborneol (**12**). The physical constants and spectroscopic and glc characteristics of this product were identical to those recorded for the sample of **12** isolated from *H. sativum* (*vide infra*).

*Oxidation of (−)-5-Oxoborneol (**4**) and (−)-5*-exo-*Hydroxyborneol (**5**)*

Treatment of (−)-5-oxoborneol (**4**) or (−)-5-*exo*-hydroxyborneol (**5**) with pyridinium chlorochromate (14) in methylene chloride at room temperature for 4–5 h provided (−)-bornane-2,5-dione (**6**) in ~80% yield; mp 207–209°C; $[\alpha]_D$ −113°. The product had the same ir (CCl_4), nmr (CCl_4 and C_6D_6), tlc (silica gel, petroleum ether – ether 1:1), and glc (3% SE-30, Carbowax C-20M, and 20% DEGS)[9] characteristics as authentic (+)-bornane-2,5-dione prepared from (+)-camphor.

Oxidation of 6-endo-*Hydroxyborneol (**9**)*

Treatment of 6-*endo*-hydroxyborneol (**9**) (11 mg) with pyridinium chlorochromate in CH_2Cl_2 (14) for 6 h gave, after preparative glc (20% DEGS), bornane-2,6-dione (**10**) as a colourless solid (31% yield) with the same spectroscopic and glc characteristics as authentic bornane-2,6-dione prepared from (+)-camphor (*vide infra*).

(+)-Bornane-2,5-dione and Bornane-2,6-dione

Oxidation of (+)-camphor with chromium trioxide in acetic acid – acetic anhydride according to the literature procedure (15) provided a mixture of bornanediones (~7% yield) which were separated by preparative glc (30% SE-30).[10]

Bornane-2,6-dione (**10**) of shorter retention time, crystallised

[10]10% Carbowax C-20M resolves 2,3-, 2,5-, and 2,6-bornanediones; 20% DEGS separates 2,6-dione from the 2,3- and 2,5-diones; and 3% SE-30 separates 2,5-dione from the 2,3- and 2,6-diones.

from hexane, mp 190–192°C (sealed capillary) (lit. (15) mp 194–195°C, lit. (16) mp 192–193°C); $[\alpha]_D$ +0.7° (*c* 2.59, $CHCl_3$); ν_{max} (CCl_4): 1770, 1735 cm^{-1}; δ ($CDCl_3$): 0.95 (3H, s), 0.99 (6H, s); δ (C_6D_6): 0.48 (6H, s), 0.97 (3H, s) 1.80 (1H, t, J = 5 Hz, C(4)-H); *m/e* (relative intensity): 166(M^+, 96), 123(38) 109(38), 97(61), 95(48), 83(40), 81(52), 69(65), 41(100), 39(52).

(+)-Bornane-2,5-dione (enantiomer of **6**) crystallised from hexane, mp 210–211°C (sealed capillary) (lit. (7*c*) mp 213–214°C); $[\alpha]_D^{26}$ +115° (*c* 1.71, $CHCl_3$); ν_{max} (CCl_4): 1755 cm^{-1}; δ ($CDCl_3$): 0.97 (3H, s), 1.05 (6H, s); *m/e* (relative intensity): 166(M^+, 93), 123(54), 95(37), 83(59), 81(31), 69(78), 67(23), 53(25), 41(100).

Fermentation with H. sativum

Helminthosporium sativum[11] was grown in 1-L Erlenmeyer flasks each containing modified Czapek-Dox medium (200 mL) (water (200 mL), sucrose (6 g), sodium nitrate (0.4 g), yeast extract (0.2 g), potassium dihydrogen phosphate (0.2 g), potassium chloride (0.1 g), magnesium sulphate heptahydrate (0.1 g), iron(III) (0.1 mg), zinc(II) (0.1 mg), manganese(II) (0.05 mg), and copper(II) (0.1 mg)) on a rotary shaker (106 rpm) at 26°C. After 3 days (+)- or (−)-bornyl acetate was added (0.2 g per flask). After a further 7–10 days the mycelium was separated by filtration and the culture filtrate extracted with ether, using a continuous extraction apparatus, for 3 days. The ether extract was dried (Na_2SO_4) and evaporated to provide the crude fermentation product.

Isolation of Hydroxyborneols ***5***, ***20***, ***21***, *and* ***12***

The crude fermentation product (2.0 g) from fermentation with (−)-bornyl acetate (~2 g) as substrate was purified by column chromatography on silicic acid (50 g). After elution of the less strongly absorbed material (0.4 g) with chloroform, the hydroxyborneols were removed as a mixture (1.2 g) with 3% methanol in chloroform. A portion of this mixture of hydroxyborneols (0.5 g) was purified further by several cycles of column chromatography (silica gel, deactivated to grade III, 100:1 ratio of adsorbant to substrate, eluted with ether – petroleum ether 1:1 then 2:1). This provided fractions that were sufficiently pure in each of the four hydroxyborneols for them to be purified by recrystallisation from benzene.

(−)-5-exo-*Hydroxyborneol* (**5**) (135 mg), mp 257–259°C (sealed capillary); $[\alpha]_D^{27}$ −15.9° (*c* 0.94, $CHCl_3$), identical to previously characterised 5-*exo*-hydroxyborneol (**5**) (*vide supra*) by nmr ($CDCl_3$), ir ($CHCl_3$ and Nujol mull), mass spectrometry as well as by tlc and mixture melting point.

(−)-5-endo-*Hydroxyborneol* (**20**) (37 mg), mp 244.5–245.5°C (sealed capillary); $[\alpha]_D^{25}$ −33.25° (*c* 0.8, $CHCl_3$); ν_{max} ($CHCl_3$): 3600, 3450, 1060 cm^{-1}; δ ($CDCl_3$): 0.82 (3H, s, CH_3), 0.91 (6H, s, CH_3), 1.6–1.85 (4H, complex), 1.9–2.4 (1H, complex), 4.06 (1H, d of d, J = 4 and 10 Hz, 2-*exo*-H), 4.44 (1H, complex, 5-*exo*-H); *m/e* (relative intensity): 170(M^+, 30), 152(30), 137(63), 111(50), 109(100), 108(84), 95(31), 41(27), 32(23). *Anal.* calcd. for $C_{10}H_{18}O_2$: C 70.55, H 10.66; found: C 70.73, H 10.60.

(−)-6-exo-*Hydroxyborneol* (**21**) (18 mg), mp 268–270.5°C (sealed capillary); $[\alpha]_D^{25}$ −56.4° (*c* 0.96, CH_3CN); ν_{max} ($CHCl_3$): 3600, 1070, 1050 cm^{-1}; δ ($CDCl_3$): 0.82, 0.95, 1.03 (3H each, s, CH_3), 1.6–1.95 (3H, complex), 2.0–2.4 (1H, complex), 4.04 (1H, d of d, J = 10 and 4 Hz, 2-*exo*-H) 4.33 (1H, m, 6-*endo*-H); *m/e* (relative intensity): 170(M^+, 1), 111(17), 109(41), 108(100), 95(16), 93(18), 55(13), 43(18), 41(23). *Anal.* calcd. for $C_{10}H_{18}O_2$: C 70.55, H 10.66; found: C 70.41, H 10.50.

[11]Subculture from single spore of culture isolated by Dr. Stanley Chinn (Canada Agricultural Research Station, Saskatoon, Sask.) from wheat (subculture sent to ATCC).

(−)-3-exo-*Hydroxyborneol* (**12**) (17 mg), mp 247–240°C (sealed capillary); $[\alpha]_D^{25}$ −19.4° (*c* 0.66, $CHCl_3$); ν_{max} ($CHCl_3$): 3650, 3470, 1060, 1050 cm^{-1} (cf. ref. 8*b*); δ ($CDCl_3$): 0.86, 0.89, and 1.09 (3H each, s, CH_3), 1.65–2.0 (3H, complex), 3.55 (1H, d, J = 2Hz, 3-*endo*-H), 3.97 (1H, m, 2-*exo*-H); the nmr spectrum in pyridine-d_5 with a trace of D_2O is identical to published spectrum (8*a*); *m/e* (relative intensity): 170(M^+, 3), 152(31), 123(18), 111(67), 109(32), 108(35), 95(100), 81(29), 69(25), 60(18), 55(18), 43(22), 41(28), 32(19), 31(18). *Anal.* calcd. for $C_{10}H_{18}O_2$: C 70.55, H 10.66; found: C 70.41, H 10.76.

5-exo-, *5*-endo-, *6*-exo-, *and 3*-exo-*Acetoxybornyl Acetates*

Hydroxyborneol **5**, **20**, **21**, or **12**, (5–15 mg) was stirred with pyridine – acetic anhydride (4:1, 2 mL) at 90°C under nitrogen for 18–21 h. After dilution with water (10 mL) and extraction with ether (3 × 5 mL), the ether extracts were washed successively with excess 1 *N* aqueous hydrochloric acid, water, excess saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride. Removal of solvent provided the appropriate acetoxybornyl acetate in almost quantitative yield.

5-exo-*Acetoxybornyl acetate* (**7**) was identical by tlc (silica gel, ether – petroleum ether 1:1) and glc (3% SE-30 and 20% DEGS) as well as nmr ($CDCl_3$), ir (CCl_4), and mass spectrometry to material isolated from chemical oxidation of bornyl acetate (*vide supra*).

5-endo-*Acetoxybornyl acetate* derived from **20**; ν_{max} (CCl_4): 1740, 1240 cm^{-1}; δ ($CDCl_3$): 0.83 (3H, s, CH_3), 0.97 (6H, s, CH_3), 2.07 and 2.10 (3H each, s, CH_3CO_2), 4.94 (1H, d of d, J = 9 and 3.5 Hz, 2-*exo*-H), 5.15 (1H, m, 5-*exo*-H); *m/e* (relative intensity): 254(M^+, 21), 195(73), 152(32), 137(27), 135(40), 134(27), 119(48), 109(53), 108(41), 93(43), 43(100), 32(25).

6-exo-*Acetoxybornyl acetate* derived from **21**; ν_{max} (CCl_4) 1740, 1235, 1060, 1040 cm^{-1}; δ ($CDCl_3$): 0.89, 0.93, and 1.03 (3H each, s, CH_3), 2.05 and 2.08 (3H each, s, CH_3CO_2—), 4.99 (1H, d of d, J = 10 and 3.5 Hz, 2-*exo*- or 6-*endo*-H), 5.31 (1H, d of d, J = 7.5 and 4 Hz, 2-*exo*- or 6-*endo*-H); *m/e* (relative intensity): 254(M^+, 15), 212(6), 197(6), 194(5), 152(14), 134(9), 119(12), 109(16), 108(100), 95(6), 93(9), 43(34), 41(7), 32(16).

3-exo-*Acetoxybornyl acetate* derived from **12**; ν_{max} (CCl_4): 1740, 1240, 1045 cm^{-1}; δ ($CDCl_3$): 0.87, 0.88, and 1.08 (3H each, s, CH_3) 2.06 and 2.10 (3H each, s, CH_3CO_2), 4.50 (1H, d, J = 3 Hz, 3-*endo*-H), 5.21 (1H, m, 2-*exo*-H); *m/e* (relative intensity): 254(M^+, 4), 152(51), 137(22), 135(28), 134(44), 124(10), 123(23), 119(29), 109(20), 108(13), 102(24), 96(10), 95(48), 83(14), 81(12), 80(22), 43(100), 41(14).

Oxidation of (−)-5-endo-*Hydroxyborneol (**20**)*

Treatment of 5-*endo*-hydroxyborneol (**20**) (8 mg) with pyridinium chlorochromate (14) for 6 h gave (−)-bornane-2,5-dione as a colourless solid (6 mg, 76% yield); nmr (in CCl_4 and C_6D_6), ir (CCl_4), tlc (silica gel, ether – petroleum ether 1:1), and glc (3% SE-30, 10% Carbowax C-20M, and 20% DEGS) characteristics identical to those of authentic bornane-2,5-dione (*vide supra*).

Oxidation of 6-exo-*Hydroxyborneol (**21**)*

Treatment of 6-*exo*-hydroxyborneol (**21**) (10 mg) with pyridinium chlorochromate (14) for 6 h gave bornane-2,6-dione (**10**) as a colourless solid after preparative glc purification (20% DEGS); nmr ($CDCl_3$ and C_6D_6), ir (CCl_4) spectra of this specimen of **10** were identical to those recorded for the authentic compound prepared from camphor (*vide supra*).

*Hydroxylation of (+)-Bornyl Acetate (**22**) by* H. sativum

The reaction product from fermentations using (+)-bornyl acetate (**22**) as substrate was acetylated and then analysed by comparing the nmr and glc characteristics with the diacetates derived from hydroxyborneols **5**, **20**, **21**, and **12** (*vide supra*).

The results obtained from four experiments indicated that the original microbiological product was a mixture of (+)-5-*exo*-hydroxyborneol (**23**), (+)-5-*endo*-hydroxyborneol (**24**), and (+)-3-*exo*-hydroxyborneol (**25**). The yields of diols from four separate fermentations are shown in Table 3.

Quantitative Gas–Liquid Chromatographic Analysis of H. sativum *Products*

Analysis of Fermentation Extracts Directly

This was performed on a Varian Aerograph model 90-P chromatograph fitted with TC detector and analytical 20% DEGS column, using 10–100 μL injections of solutions in ethyl acetate. With a column oven temperature of ~130°C the absolute quantity of hydroxyborneols was determined by injecting known quantities of the fermentation products and comparing the area of the peak corresponding to the hydroxyborneols with that arising from the injection of known quantities of pure 5-*exo*-hydroxyborneol as a standard. In the same way, using a column oven temperature of ~70°C and (−)-bornyl acetate as standard, it was possible to determine the absolute quantities of bornyl acetate and borneol. All injections were performed several times to ensure reproducibility of the results, and fermentation peak areas were only compared with those of standards that had been injected immediately beforehand.

Analysis of Acetylated Fermentation Extracts

Fermentation extracts were acetylated in the usual way and the acetylated products were analysed by glc (analytical 3% SE-30 column at ~100°C). The absolute quantities of acetoxybornyl acetates were determined by using 5-*exo*-acetoxybornyl acetate (**13**) as a standard, taking the same precautions that are described above. The identity of the peaks in the glc analysis was confirmed by isolation (preparative glc, 30% SE-30) and nmr analysis of the isolated components.

Fermentation with F. culmorum

Fusarium culmorum[12] was grown in 4-L Erlenmeyer flasks each containing Raulin–Thom medium (1 L) (water (1 L), glucose (50 g), tartaric acid (2.7 g), ammonium tartrate (2.7 g), ammonium phosphate $NH_4H_2PO_4$ (0.4 g), potassium carbonate (0.4 g), magnesium carbonate 0.27 g), ammonium sulphate (0.17 g), zinc sulphate heptahydrate (0.047 g), and ferrous sulphate heptahydrate (0.047 g)) on a rotary shaker (120 rpm) at 26°C. After 7 days, (+)- or (−)-bornyl acetate was added (1.0 g per flask). After a further 18 days the mycelium was separated by filtration and the culture filtrate extracted with ether using a continuous extraction apparatus for 3 days. The ether extract was dried (Na_2SO_4) and evaporated to provide the crude fermentation product.

*Isolation of 5-*exo*-Hydroxybornyl Acetate (**26**)*

The crude fermentation product (3.01 g) was purified by column chromatography (silicic acid, 180 g). Elution with $CHCl_3$ provided 5-*exo*-hydroxybornylacetate (**26**) (900 mg; 97% pure by glc); ν_{max} ($CHCl_3$): 3600, 1720, 1250, and 1040 cm^{-1}; δ ($CHCl_3$): 0.84, 0.86, 1.09 (3H each, s, CH_3), 1.99 (3H, s, CH_3CO_2—) 3.84 (1H, d of d, J = 7.5 and 3 Hz, 5-*endo*-H), 4.76 (1H, m, 2-*exo*-H).

*5-*exo*-Acetoxybornyl Acetate (**7**)*

5-*exo*-Hydroxybornyl acetate (**26**) (41 mg), isolated in 96% purity (glc, 10% Carbowax C-20M TPA) from silicic acid chromatography of the crude fermentation product, was stirred at 90°C under a nitrogen atmosphere with pyridine – acetic anhydride (4:1, 2 mL) for 22 h. The reaction mixture was diluted with water (10 mL), extracted with ether (3 × 5 mL), and the ether layers washed with excess aqueous 1 *N* hydrochloric acid, water, excess saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride. Removal of solvent provided 5-exo-*acetoxybornyl acetate* (**7**), identical by tlc (silica gel, ether – petroleum ether 1:2), glc (3% OV-17 and 10% Carbowax C-20M; HP-5831A), and by nmr ($CDCl_3$) and ir ($CHCl_3$) spectrometry to material prepared previously by lithium trimethoxy aluminum hydride reduction of 5-oxobornyl acetate (**3**) followed by acetylation (*vide supra*).

*Oxidation of 5-*exo*-Hydroxybornyl Acetate (**26**)*

Treatment of 5-*exo*-hydroxybornyl acetate (**26**) (46 mg) with pyridinium chlorochromate (14) for 7 h gave 5-oxobornyl acetate (**3**) (41 mg), identical by tlc (silica gel, ether – petroleum ether 1:2) and glc (3% OV-17 and 10% Carbowax C-20M TPA; HP-5831A) and by nmr ($CDCl_3$) and ir ($CHCl_3$) spectrometry with authentic material isolated from the oxidation of bornyl acetate with chromium trioxide – acetic acid (*vide supra*).

Quantitative Gas–Liquid Chromatographic analysis of F. Culmorum *Products*

Quantitative glc analysis of the fermentation products of (+)- and (−)-bornyl acetate was performed using (−)-camphorquinone as an added internal standard. The relative detector responses to borneol, bornyl acetate, 5-*exo*-hydroxybornyl acetate, and (−)-camphorquinone were determined by injection of a mixture of known quantities of the four substrates (10% Carbowax C-20M TPA; HP-5831A); an average of three determinations was taken. By injection of a mixture of a known quantity of the fermentation product and a known quantity of camphorquinone (10% Carbowax C-20M TPA; HP-5831A) the relative peak areas, after correction for the detector response, were used to determine the absolute quantities of borneol, bornyl acetate, and 5-*exo*-hydroxybornyl acetate; an average of two determinations was taken.

[12]Subculture of culture No. CBS 171.28 obtained from Centraal-bureau voor Schimmelcultures, Baarn, Holland.

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