Development of an Enzyme-Linked Immunosorbent Assay for Geosmin

Si-Yin Chung,*,† John R. Vercellotti,† Peter B. Johnsen,† and Phillip H. Klesius‡

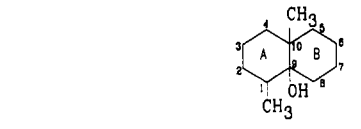
Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 19687, 1100 Robert E. Lee Boulevard, New Orleans, Louisiana 70179, and Animal Parasite Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 952, Wire Road at Simms Drive, Auburn, Alabama 36830

A direct competitive ELISA for geosmin (a musty/earthy off-flavor compound) was developed. Antibodies were produced in goats injected with a BSA–argosmin C (a dehydration product of geosmin) conjugate. The sensitivity and specificity of the antibody were respectively determined in a microtiter plate coated with the purified antibody which was subsequently detected with an alkaline phosphatase–argosmin C conjugate. The antibody had the most cross-reactivity with an argosmin C related compound, namely, 4,4a,5,6,7,8-hexahydro-4a-methyl-2(3*H*)-naphthalenone. It cross-reacted equally with geosmin, 2-ketogeosmin, and 2-decalone but had limited recognition for norbornane, indicating that the binding of the antibody was restricted mainly to the bicyclic structure (A and B rings) of geosmin. Because it had some cross-reactivity with 2-methylisoborneol (MIB), it was assumed that the methyl group in geosmin might also play a role in antibody recognition. In addition, the presence of two OH groups on the A and B rings of 1,5-decalindiol also had an effect on the antibody binding. The assay had a sensitivity of 1 μg/mL.

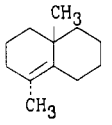
INTRODUCTION

Two off-flavor compounds that have long been known as the cause of earthy/musty odor problems in drinking water (Aoyama, 1990; Yagi, 1988; Burlingame et al., 1986; Izaguirre et al., 1982; McGuire et al., 1981) and in fish (Martin et al., 1987; Lovell, 1983; Yurkowski and Tabachek, 1980) have been identified as geosmin (1) (1,10-dimethyl-9-decalol) and 2-methylisoborneol (MIB). These compounds are produced as metabolites by microorganisms such as actinomycetes and blue-green algae (Maga, 1987; Sugiura et al., 1986; Gerber, 1983; Tabachek and Yurkowski, 1976; Medsker et al., 1968). Fish producers and water suppliers are most affected by the off-flavor problem because of their inability to market their fish crop and water when desired. Processors need to do a thorough job of screening and taste-testing fish and water for flavor quality to maintain standards. While gas chromatography can be used to determine the flavor status of fish and water, the method requires extensive sample cleanup procedures and expensive equipment and is time-consuming. A technique such as an enzyme-linked immunosorbent assay (ELISA) can be used as an alternative to detect the off-flavors. ELISA offers considerable advantage over gas chromatography in terms of cost, time, simplicity, ease of sample preparation, and minimal training. Recently an ELISA for MIB has been developed (Chung et al., 1990).

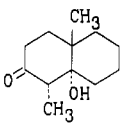
In an attempt to develop an ELISA for geosmin, derivatives of geosmin were synthesized. Of these 2-ketogeosmin (3) was made with the desire that a 2-ketogeosmin *O*-(carboxymethyl)oxime (4) could be obtained for subsequent coupling with bovine serum albumin (BSA) to form a BSA–geosmin conjugate. However, isolation of the oxime was unsuccessful. Alternatively, an oxime derivative of argosmin C, namely, 2-ketoargosmin C *O*-(carboxymethyl)oxime (5), was isolated. Argosmin C (2) is known to be a dehydration product of geosmin (Gerber, 1968; Rosen, 1968).



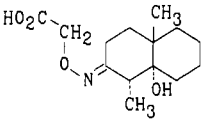
1



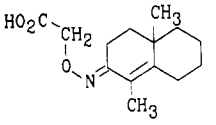
2



3



4



5

This study reports the feasibility of using polyclonal antibodies raised against a BSA–argosmin C conjugate to develop an ELISA for geosmin.

MATERIALS AND METHODS

Materials. Chemicals purchased from Aldrich Chemical Co. (Milwaukee, WI) included 2-methylcyclohexanone, 1-chloro-3-pentanone, lithium, carboxymethoxylamine hemihydrochloride, norbornane, 2-decalone, 1,5-decalindiol, and 4,4a,5,6,7,8-hexahydro-4a-methyl-2(3*H*)-naphthalenone. Ammonia gas was obtained from Matheson Gas Products (LaPorte, TX). Bovine serum albumin (BSA), Tris-buffered saline (TBS), alkaline phosphatase, *p*-nitrophenyl phosphate, rabbit antigoat IgG–horseradish peroxidase, and *o*-phenylenediamine dihydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Protein G Acti-Disk (47-mm matrix diameter; matrix thickness 0.55 mm, pore 1 μm) was obtained from FMC BioProducts (Rockland, ME). Precast polyacrylamide gels were purchased from Schleicher & Schuell

† Southern Regional Research Center.
‡ Animal Parasite Research Laboratory.

(Keene, NH). Microtiter plates of Immulon II and plate reader M700 were purchased from Dynatech Laboratories, Inc. (Chantilly, VA). Geosmin was obtained from Givaudan Co. (Paramus, NJ). MIB was synthesized in our laboratory according to the method of Wood and Snoeyink (1977).

^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectra were obtained at 90 and 360 MHz, respectively, with a Bruker AM-360 spectrometer at 300 K using ca. 15 mg of sample in 0.5 mL of $CDCl_3$ in all experiments except the long-range C/H coupling experiment in which a solution of ca. 45 mg in 0.5 mL of $CDCl_3$ was used. Assignments of chemical shifts are based on ^{1}H, COSY, decoupling, NOE, ^{13}C, DEPT, and HETCOR NMR spectroscopy. Protons in ring A are labelled β (cis to C_{10}–CH_3) and α (trans to C_{10}–CH_3). Protons in ring B, which presumably exists in an approximate chair form, are labeled a (axial) and e (equatorial). Infrared (IR) spectra were obtained on a Bio-Rad Digilab FTS 40 Fourier transform infrared spectrophotometer. Mass spectra (MS) (direct probe or GC/MS) were obtained at 70 eV on a Finnegan 4500 mass spectrometer with Incos data system. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Thin-layer chromatography (TLC) on silica gel 60 was developed with ethyl acetate/methanol/ammonium hydroxide (65:35:11) and visualized with iodine vapors. Scanning UV spectrophotometry was carried out on a Gilford response instrument.

Synthesis of 2-Ketogeosmin (3). *1,10-Dimethyl-1(9)-octal-2-one* (**2b**). **2b** was prepared with some modification of the method of Zoretic et al. (1975). Briefly, a mixture of 2-methylcyclohexanone (**2a**) (28.46 mL), 1-chloro-3-pentanone (52.72 mL), and concentrated sulfuric acid (0.55 mL) in benzene (110 mL) was refluxed for 18 h and then cooled to room temperature. A solution of diluted sodium bicarbonate (60 mL of a mixture of saturated bicarbonate and water in 1:1 ratio) was added. The resulting mixture was concentrated by evaporating the benzene and extracting three times with ether (50 mL each). The ether extract was washed with 100 mL of water, dried over magnesium sulfate, filtered, and concentrated. The residue was distilled in vacuo (water bath at 50–90 °C) to give the first fraction (32 °C, 0.17 mm), following which an oil bath (140 °C) was used to distill the remainder of the residue, thus giving the second fraction: bp 90 °C, 0.2 mmHg (ref bp 89 °C); MS m/z = 178.

1,10-Dimethyl-1(9)-epoxyoctal-2-one (**2c**). **2c** was prepared according to the method of Ayer et al. (1976). Briefly, to a solution of **2b** (13.4 g) in methanol (303 mL) at 0 °C were added dropwise simultaneously 30% hydrogen peroxide (24.24 mL) and 4 N NaOH (11.71 mL). The mixture was stirred at 0 °C for 3 h and then allowed to stand for 1 day at 0 °C and 3 days at room temperature. The methanol in the resulting mixture was removed by evaporation under reduced pressure. Water (50 mL) was added to the residue, and extracted three times with ether (50 mL each). The ether extract was washed with 100 mL of water, dried over magnesium sulfate, filtered, and concentrated. The residue was distilled in vacuo by use of an oil bath (135 °C) to give an oily mixture (12.1 g, 83%): bp 91–94 °C, 0.15 mmHg (ref bp 90–93 °C); MS m/z = 194.

2-Ketogeosmin (**3**). **3** was prepared with some modification of the method of Ayer et al. (1976). Briefly, liquid ammonia (600 mL) was distilled through sodium hydroxide to a chilled 3-neck round-bottom flask containing **2c** (8.8 g). Lithium (2.0 g) was added, and the resulting mixture (blue) was allowed to stir for 6 h (a dry ice condenser refluxed the liquid ammonia). Excess ammonium chloride (40 g) was added until the blue color disappeared. The ammonia was then allowed to evaporate. Water (100 mL) was added and extracted three times with ether (50 mL each). The ether extract was washed with 100 mL of water, dried over magnesium sulfate, filtered, and concentrated, leaving a slightly turbid yellow oil (8.1 g) which solidified on standing overnight at below 0 °C. Crystallization from hexane gave **3** (2.5 g, 56%): mp 109–110 °C (ref 109–110 °C); MS m/z = 196; IR ($CHCl_3$) 3482, 1700 cm^{-1} (ref 3600, 1720 cm^{-1}).

Synthesis of 2-Ketoargosmin C *O*-(Carboxymethyl)oxime (5). The oxime **5** was prepared with some modification of the method of Chung et al. (1990). Briefly, a mixture of 2-ketogeosmin (**3**) (0.447 g) in ethanol (10 mL) and carboxymethoxylamine hemihydrochloride (1.115 g) in 2 N NaOH (7.6 mL) was heated at 45 °C for 30 min, allowed to stand overnight at room temperature, and concentrated by evaporating the ethanol with nitrogen gas. Water (50 mL) was then added, and the pH was adjusted to 9.4 with approximately 6 mL of 1 N NaOH. The solution was washed twice with 40 mL of ethyl acetate. The aqueous layer was then adjusted to pH 4.7 with about 6 mL of 1 N HCl. The precipitate thus obtained was washed with 500 mL of cold water and dried in vacuo with anhydrous calcium sulfate. The resultant **5** gave the following NMR data in addition to those indicated in Table I: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 3.01 (αH, C-3), 2.28 (βH, C-3), 1.65 (βH, C-4), 1.5 (αH, C-4), 1.65 (C-5, eH), 1.25 (C-5, aH), 1.6 (C-6, a and e H's), 1.85 (C-7, eH), 1.28 (C-7, aH), 2.73 (C-8, eH), 1.95 (C-8, aH), 1.79 (C-1 CH_3, doublet), 1.08 (C-10, CH_3), 4.63 (OCH_2), 10.5 ($-CO_2H$). Coupling constants (J) are apparent first-order values. A doublet exists for the methyl group at C-1 with $J_{CH3,H\text{-}8a}$ = 1.2 Hz. Signals for the C-3 protons are well separated from those of other protons, and each proton displays the eight lines expected for an ABXY system: J_{gem} = 17.6, $J_{3\alpha,4\beta}$ = 3.2, $J_{3\alpha4\alpha}$ = 4.7, $J_{3\beta4\beta}$ = 5.9, $J_{3\beta4\alpha}$ = 13.6 Hz. The J_{gem} of H-8 and J_{vic} of H-8a and the H-7 protons were analyzed through coupling constants derived from a 500-MHz spectrum in which the H_{8a} and H_{7e} signals do not overlap: J_{gem} = 19.1, $J_{8a,7a}$ = 14.3, $J_{8a,7e}$ = 4.8 Hz; ^{13}C NMR ($CDCl_3$, 90.556 MHz) 121.27 (C-1), 160.44 (C-2), 19.64 (C-3), 36.49 (C-4), 41.90 (C-5), 21.70 (C-6), 27.00 (C-7), 26.67 (C-8), 151.90 (C-9), 35.38 (C-10), 12.20 (C_1–CH_3), 22.69 (C_{10}–CH_3), 70.15 (OCH_2), 173.54 (CO_2H).

Table I. Characteristics of 2-Ketoargosmin C *O*-(Carboxymethyl)oxime

method	2-ketoargosmin C *O*-(carboxymethyl)oxime
MS m/z	251 (67), 176 (64), 160 (94), 91 (85), 79 (71)
IR ($CHCl_3$), cm^{-1}	3000–2500, 1738, 1700, 1600, 1247
NMR ($CDCl_3$)	see text for details
elemental analyses	C, 66.93; H, 8.37; N, 5.57 (calcd for $C_{14}H_{21}O_3N$) C, 67.12; H, 8.4; N, 5.45 (found)
UV max, nm	252
mp, °C	121–123
R_f (TLC)	0.6

Preparation of BSA–Argosmin C Conjugate. 2-Ketoargosmin C *O*-(carboxymethyl)oxime (**5**) was coupled to BSA according to the mixed anhydride method as described by Chung et al. (1990). The resultant BSA–argosmin C conjugate was characterized by means of UV spectral analysis.

Immunization and Test for Presence of Antibodies in Serum. Immunization of goats with the BSA–argosmin C conjugate and test in ELISA were respectively performed according to the method of Chung et al. (1990). A microtiter plate (Immulon II, Dynatech) coated with an ovalbumin–argosmin C conjugate (prepared in the same way as the BSA–argosmin C conjugate) was used in the ELISA to test antisera for the presence of antibodies. A rabbit antigoat IgG–horseradish peroxidase conjugate and its substrate (*o*-phenylenediamine dihydrochloride) were used as the detecting system in the ELISA.

Preparation of an Alkaline Phosphatase–Argosmin C Conjugate. The enzyme conjugate was prepared according to the mixed anhydride method. Briefly, a mixture of triethylamine (1 μL) and 2-ketoargosmin C *O*-(carboxymethyl)oxime (**5**) (1.65 mg) in tetrahydrofuran (1 mL) was cooled to −5 °C. Isobutyl chloroformate (1 μL) was added to the mixture and the resultant solution stirred for 30 min at −5 °C. The solution (0.2 mL) was then added dropwise to 2 mL of a cold solution of alkaline phosphatase (1 mg/mL) in 5% aqueous dioxane, pH 9. Sodium hydroxide was added to maintain the pH. The mixture was then stirred overnight at 4 °C, dialyzed against 0.05 M Tris buffer, pH 8.2, and 0.001 M $MgCl_2$, and stored at 4 °C.

Purification of Antibody. The antibody was purified by using a protein G Anti-Disk. Before the antiserum was applied, the disk was washed with 60 mL of PBS at a rate of 6 mL/min and equilibrated with an additional 60 mL. The antiserum was diluted 1:10 in PBS, and 10 mL of the diluted serum was recirculated through the disk at a rate of 3 mL/min for 1 h. The disk was washed with PBS (150 mL), and IgG was isolated by eluting with 0.1 M glycine hydrochloride (60 mL), pH 2.8. The eluted IgG fractions (5 mL each) were then neutralized with 1 M Tris, pH 9.6 (0.25 mL per fraction), concentrated in an Am-

icon cell (YM 30 membrane), and stored at −20 °C. The protein concentration was determined from an extinction coefficient of $E^{0.1\%}$ at 280 nm (=1.4). The purity of the antibody was confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reduced condition.

Competitive ELISA. A direct competitive ELISA between geosmin and alkaline phosphatase–argosmin C conjugate for the purified antibody adsorbed to the plate was carried out. To determine a standard curve, geosmin at a concentration ranging from 0.1 to 1000 μg/mL in 5% ethanol–TBS was used. Briefly, a solution of geosmin was incubated with an equal volume of enzyme conjugate (1:1000) in ethanol–TBS–ovalbumin (1%) at 25 °C for 5 min. The mixture (100 μL/well) was added to a microtiter plate that had been coated overnight at 4 °C with purified antibody (5 μg/mL) in carbonate buffer, pH 9.6, and blocked at 37 °C for 3 h with 0.5% gelatin. The plate was incubated for 60 min and washed three times with TBS–Tween 20 (0.05%). A substrate solution containing *o*-nitrophenyl phosphate (1 mg/mL) in 10% diethanolamine and 0.5 mM $MgCl_2$, pH 9.8, was added. After incubation in the dark at 25 °C for 2 h, the reaction was terminated by adding 50 μL of 2 N NaOH. Absorbance at 405 nm was then read in a Dynatech MR700 plate reader. A standard curve was prepared by plotting the percent of maximum absorbance versus the concentration of geosmin on semilog scale. The percent of maximum absorbance is defined as $(A_s/A_0) \times 100$, where A_s and A_0 are the absorbance obtained at each standard concentration and the absorbance of the blank in the absence of geosmin, respectively.

An indirect competitive ELISA between free geosmin and bound argosmin C for the antibody was also performed according to the procedure of Chung et al. (1990).

Determination of the Specificity of the Antibody. To determine the antibody specificity, geosmin, MIB, norbornane, and six other geosmin structurally related compounds were tested in the direct competitive ELISA. The percent cross-reactivity (CR) was then determined and defined as: CR (%) = IC_{50}(geosmin)/IC_{50}(test compound), where IC_{50}(geosmin) is the concentration of geosmin causing a 50% reduction in maximum absorbance and IC_{50}(test compound) is the concentration of test compound causing similar reduction.

RESULTS AND DISCUSSION

Characterization of 2-Ketoargosmin C *O*-(Carboxymethyl)oxime (5). In an attempt to prepare 2-ketogeosmin *O*-(carboxymethyl)oxime (4), 2-ketogeosmin (3) was synthesized (Figure 1) and allowed to react with carboxymethoxylamine (Figure 2). Attempts to isolate 4 from the reaction mixture were unsuccessful, and instead, 2-ketoargosmin C *O*-(carboxymethyl)oxime (5) was obtained (Figure 2). It was thought that the reason 4 could not be isolated was because it had changed to 5 as a result of pH adjustment with HCl during isolation. Gerber (1983, 1968) and Rosen (1968) have reported that acid treatment of geosmin (1) could lead to the formation of argosmin C (2), a dehydration product of 1.

The existence of 5 was confirmed by means of MS, IR, NMR, and elementary analyses (Table I). Although a mass spectrum similar to that of 5 may be exhibited by 4 due to very little molecular ion given by the tertiary alcohol in 4, the absence of OH in IR and NMR data proved that the mass spectrum was related to 5 and not 4. The IR spectrum of 5 showed absorption expected for a carboxylic acid (3000–2500, 1738, and 1247 cm^{-1}) and double bonds (1700 and 1600 cm^{-1}). By use of scanning UV spectrophotometry, 5 in an ethanol/water solution (1:1) gave a peak maximum at 252 nm with a molar absorptivity of 23 800. On TLC 5 migrated with an R_f value of 0.6 compared to R_f 0.95 observed with 3.

Spectral data from NMR studies (see Materials and Methods) indicated that 5 is a single isomer that must have either *Z* or *E* isomer structure **5a** or **5b** (Figure 3a). When the *Z*/*E* isomerism of the oxime was assigned, **5a** was a priori deemed to be the more likely isomer. There is less steric crowding in **5a**; in general, when both *Z* and *E* isomers are formed, the less crowded isomer predom-

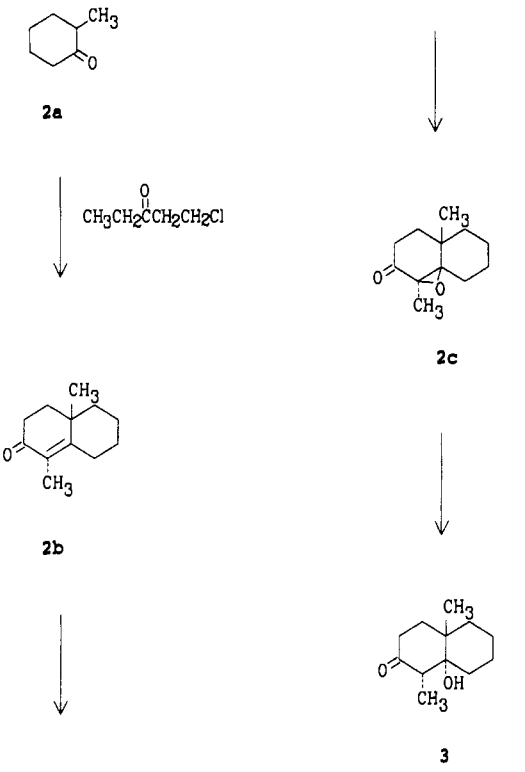


Figure 1. Steps in the preparation of 2-ketogeosmin (3).

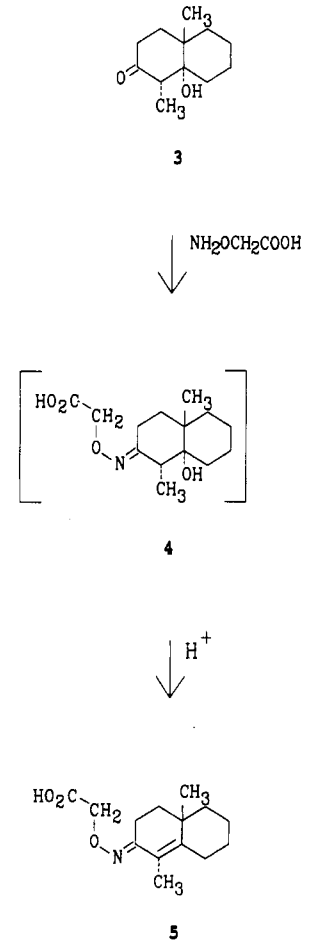


Figure 2. Preparation of 2-ketoargosmin C *O*-(carboxymethyl)-oxime (5).

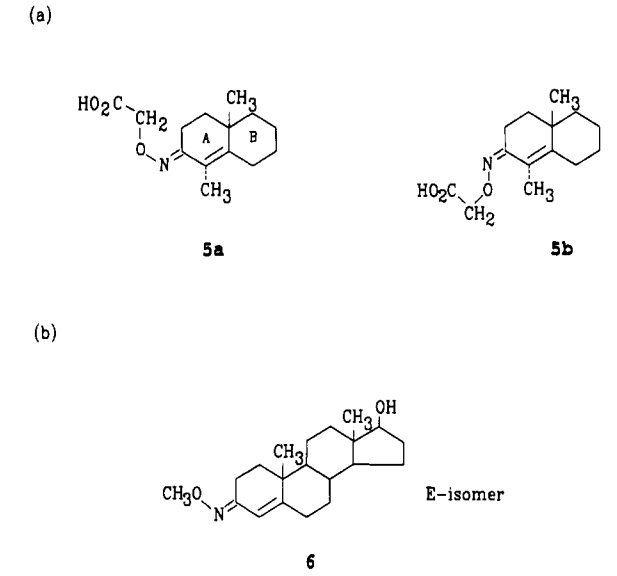


Figure 3. (a) Isomers of 2-ketoargosmin C *O*-(carboxymethyl)-oxime (**5a,b**). (b) *E* isomer of testosterone *O*-methyloxime (**6**).

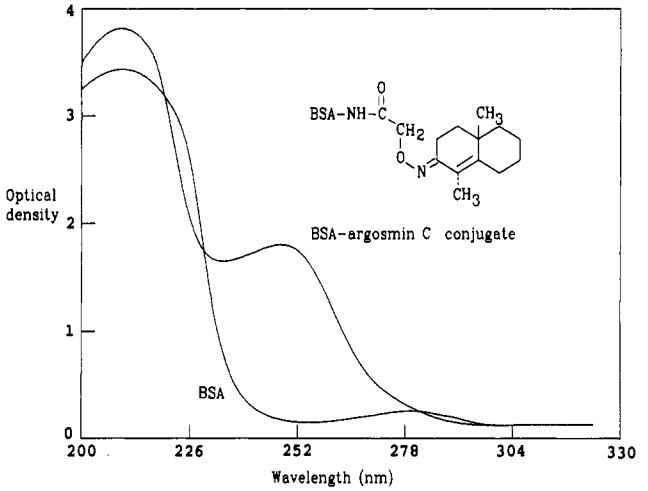


Figure 4. Comparison of the UV absorption of BSA and BSA–argosmin C conjugate.

inates. Irradiation at OCH_2 in **5a** results in a slight (0.3%) enhancement of only the signal due to H-3α (trans to the C_{10}–CH_3 group). The chemical shift of ^{13}C-3 (19.6 ppm) is similar to the corresponding signal in (*Z*)-2-butanone oxime (21.5 ppm) (Silverstein et al., 1981); this signal is at lower field (29.0 ppm) in the *E* isomer. Structure **5a** is established by comparison with literature data of model compounds, the *O*-methyloximes of testosterone (**6**) (Figure 3b): (a) the *E* isomer has a UV maximum at 251 nm (16 200), while the *Z* isomer has a UV maximum at 259 (2500) (Hara et al., 1967). Replacement of H-1 by a CH_3 group should shift the maxima to longer wavelengths by about 5 nm. (b) By use of the numbering of **3** for the testosterones, at 100 MHz the *E* isomer shows ^1H NMR signals at 3.0 ppm (dt, J = 18.5 and 4.8 Hz) for H-3α; H-3β overlaps with H-8a and H-8e signals (2.0–2.4 ppm). In the *Z* isomer the H-3 and H-8 protons all resonate at 2.1–2.4 ppm (Oka and Hara, 1968).

Characterization of BSA–Argosmin C Conjugate. The formation of the BSA–argosmin C conjugate could be confirmed by scanning the conjugate in the ultraviolet at 252 nm, where 2-ketoargosmin C *O*-(carboxymethyl)oxime (**5**) gave a UV maximum. As shown in Figure 4, a peak corresponding to **5** was observed. This peak, not seen in the control (i.e., BSA), indicated that **5** had been bound to the BSA. The molar ratio of BSA to argosmin C was found to be 1:15 according to the method of Erlanger et al. (1957).

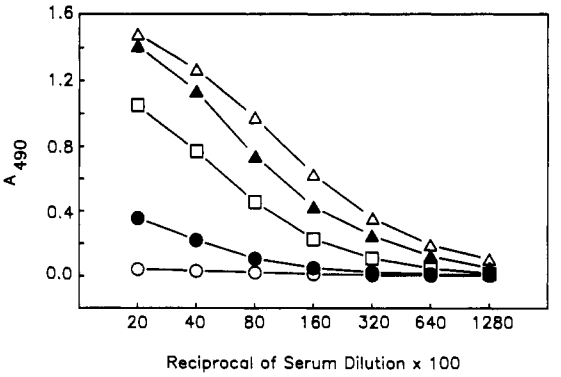


Figure 5. Titration of goat antisera. Preimmune serum (○) or antiserum drawn at week 3 (●), 4 (△), 6 (▲), or 8 (□) was serially diluted and assayed in a microtiter plate coated with ovalbumin–argosmin C conjugate. Antibodies were detected with a rabbit antigoat IgG–horseradish peroxidase conjugate.

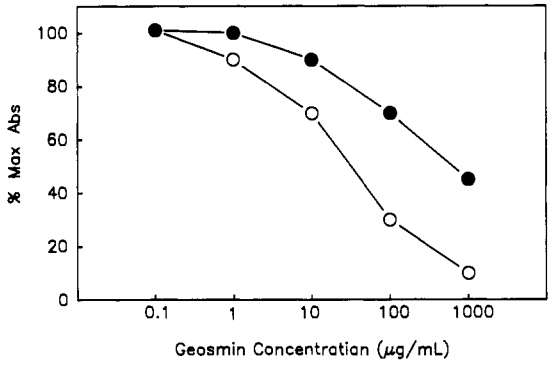
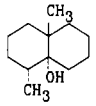
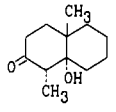
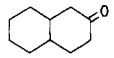
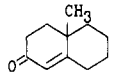
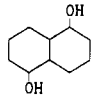
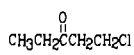
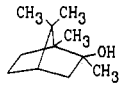


Figure 6. Standard curves for geosmin. (○) Direct competitive ELISA. Microtiter plates were coated with purified antibodies which were detected with an alkaline phosphatase–argosmin C conjugate. (●) Indirect competitive ELISA. Plates were coated with an ovalbumin–argosmin C conjugate, followed by incubation with antibodies which were detected with an antigoat IgG–horseradish peroxidase conjugate.

Detection of Antibodies in Goat Serum. The presence of antibodies in goat serum was demonstrated by comparing the titration curves of both preimmune serum and antisera (Figure 5). While no antibodies were detected in the preimmune serum, the antiserum did show a considerable increase in the antibody titer (defined as the antiserum dilution that gives an absorbance reading of 0.3 above the blank) during a 4-week period. Antibodies were detected as early as 3 weeks and increased dramatically at weak 4 following a booster injection. At week 6 the antibody titer was seen to decrease. Despite an additional booster injection, a continued decrease in the titer at week 8 was observed. For the development of an ELISA for geosmin, antiserum obtained at week 4 was used.

Assay Development. Both indirect and direct ELISAs were examined. In the indirect ELISA, the microtiter plate was coated with an ovalbumin–argosmin C conjugate and then incubated with antibodies. Detection of the bound antibodies was achieved by incubation with an antigoat IgG–peroxidase conjugate, followed by a substrate. In the case of direct ELISA, purified antibodies were adsorbed to the plate, followed by incubation with an alkaline phosphatase–argosmin C conjugate and then the substrate. Both ELISA systems applied a protein concentration of 5 μg/mL for coating in the plate. For competitive binding, a dilution of alkaline phosphatase

Table II. Specificity of Antibody toward Related Compounds

Compound	Structure	I_{50} (μg/mL)[a]	% cross-reactivity[b]
Geosmin		35.48	100
2-ketogeosmin		35.51	100
2-decalone		35.54	100
4,4a,5,6,7,8-hexahydro-4a-methyl-2(3H)-naphthalenone		19.95	178
1,5 decalindiol		251.08	14
2-methyl-cyclo-hexanone		251.19	14
1-chloro-3-pentanone		199.53	18
2-methylisoborneol		100.12	35
Norbornane		562.34	6

[a,b] See text for explanation.

conjugate or purified antibody in the direct and indirect ELISA was chosen in such a way that it gave 50–60% of the plateau activity. The standard curves thus obtained for geosmin are shown in Figure 6. A comparison of the curves indicated that the direct ELISA had a better sensitivity (1 μg/mL) than the indirect method (10 μg/mL). A similar finding was also reported by Itoh et al. (1986). On the basis of this observation, the direct ELISA was chosen for use in the following cross-reactivity study.

Specificity of Antibodies. The specificity of the antibody was determined by comparing the cross-reactivity of each related compound to that of geosmin (Table II). Geosmin had a cross-reactivity of 100%, while 4,4a,5,6,7,8-hexahydro-4a-methyl-2(3*H*)-naphthalenone had a cross-reactivity of 178%. The higher cross-reactivity for the naphthalenone may be due to the fact that it resembles argosmin C (2); the double bond in one of the rings might also contribute to higher cross-reactivity. This effect of double bond on the cross-reactivity has been reported by Abouzied et al. (1990) and Teshima et al. (1990).

Since geosmin is composed of A and B rings (see 1), it is expected that this ring structure may also play a role in the antibody recognition. To determine its role, the cross-reactivity of geosmin and that of its two precursors such as 2-methylcyclohexanone and 1-chloro-3-pentanone were compared. As can be seen in Table II, both precursors, which have one or no ring, have a very low cross-reactivity of approximately 14% as compared to 100% for geosmin. This low cross-reactivity suggests that the A and B ring structure in geosmin is important in antibody recognition and that partial elimination of the ring structure could cause a reduction in antibody binding (Stanker et al., 1989). Further evidence for the role of the rings in antibody binding came from the study on norbornane and the two other geosmin structurally related compounds, 2-ketogeosmin and 2-decalone. The latter two compounds have A and B rings with cross-reactivity similar to (i.e., 100%) that of geosmin, whereas almost no cross-reactivity was seen for norbornane with a ring structure different from that of geosmin. The only exception for A and B ring antibody recognition is 1,5-decalindiol with a cross-reactivity of 14%. This low cross-reactivity may be attributed to the presence of an OH group on each of the A and B rings (positions 1 and 5), which might hinder the binding of the antibody to the compound. Such an effect of additional side groups on antibody binding has been reported by Chung et al. (1990).

In addition to the ring structure, the antibody appeared to be able to bind to some methyl groups. This was demonstrated in the cross-reactivity study with MIB. As shown in Table II, MIB cross-reacted to some extent (35%) with the antibody. Since norbornane (i.e., the ring in MIB) had almost no cross-reactivity with the antibody, it was assumed that the cross-reactivity with MIB was due to the presence of one or more methyl groups in MIB.

Conclusion. In this study, an oxime derivative of a geosmin structurally related compound (i.e., argosmin C) was synthesized, characterized, and conjugated to BSA. Antibodies raised against the BSA–argosmin C conjugate were able to recognize geosmin in ELISA. The binding of the antibody appeared to be restricted primarily to the A and B rings in geosmin with some recognition of the methyl group. In addition, the binding capacity of the antibody could be greatly reduced if there is an OH group on each of the two rings (positions 1 and 5). The assay had a sensitivity of 1 μg/mL. Despite its inability to detect low concentrations of geosmin in water (e.g., human odor detection threshold being near 10 ng/L), the assay should find use as a means of rapid screening of geosmin under special circumstances or when the water is concentrated.

ACKNOWLEDGMENT

This paper is supported in part by a grant from the Metropolitan Water District of Southern California, La Verne, CA, and is dedicated to the memory of Leon H. Chance, who provided much valuable advice and wisdom during the course of these studies. We thank Michael S. Fisher and Timothy A. Calamari for their technical advice in the synthesis of geosmin derivatives. We also thank Elli O. Hand and Ken Belmore (both from the Department of Chemistry, University of Alabama) for identifying the 2-ketoargosmin C *O*-(carboxymethyl)oxime (5) from the NMR data. Also, we thank Terri D. Drumm and Nancy M. Morris for their assistance in GC/MS and IR spectral analyses and Anna Chromiak for her assistance in immunizing goats and collecting sera.

LITERATURE CITED

Abouzied, M. M.; Asghar, A.; Pearson, A. M.; Gray, J. I.; Miller, E. R.; Pestka, J. J. Monoclonal antibody-based enzyme-linked immunosorbent assay for C_{19}-steroids in sera of boar pigs. *J. Agric. Food Chem.* **1990**, *38*, 331–335.

Aoyama, K. Studies on the earthy-musty odours in natural water (IV). Mechanism of earthy-musty odour production of actinomycetes. *J. Appl. Bacteriol.* **1990**, *68*, 405–410.

Ayer, W. A.; Browne, L. M.; Fung, S. Metabolites of bird's nest fungi. Part 6. The synthesis of cybullol and a new synthesis of geosmin. *Can. J. Chem.* **1976**, *54*, 3276–3282.

Burlingame, G. A.; Dann, R. A.; Brock, G. L. A case study of geosmin in Philadelphia's water. *J. Am. Water Works Assoc.* **1986**, *78*, 56–61.

Chung, S. Y.; Johnsen, P. B.; Klesius, P. H. Development of an enzyme-linked immunosorbent assay using polyclonal antibodies specific for 2-methylisoborneol. *J. Agric. Food Chem.* **1990**, *38*, 410–415.

Erlanger, B. F.; Boreck, F.; Beiser, S. M.; Lieberman, S. Steroid-protein conjugates. *J. Biol. Chem.* **1957**, *227*, 713–727.

Gerber, N. N. Geosmin, from microorganisms, is trans-1,10-dimethyl-trans-9-decalol. *Tetrahedron Lett.* **1968**, 2971–2974.

Gerber, N. N. Volatile substances from actinomycetes: their role in the odor pollution of water. *Water Sci. Technol.* **1983**, *15*, 115–125.

Hara, S.; Oka, K.; Ike, Y. Quantitative resolution of *syn* and *anti* isomers of steroidal α, β-unsaturated oximes and O-methyloximes. *Chem. Ind.* **1967**, 832–833.

Itoh, Y.; Nishimura, M.; Hifumi, E.; Uda, T.; Ohtani, K.; Kawamura, O.; Nagayama, S.; Ueno, Y. Detection of ochratoxin A and T-2 toxin by one step ELISA. *Proc. Jpn. Assoc. Mycotoxicol.* **1986**, *24*, 25–29.

Izaguirre, G.; Hwang, C. J.; Krasner, S. W.; McGuire, M. J. Geosmin and 2-methylisoborneol from cyanobacteria in three water supply systems. *Appl. Environ. Microbiol.* **1982**, *43*, 708–714.

Lovell, R. T. Off-flavors in pond-cultured channel catfish. *Water Sci. Technol.* **1983**, *15*, 67–73.

Maga, J. A. Musty/earthy aromas. *Food Rev. Int.* **1987**, *3* (3), 269–284.

Martin, J. F.; McCoy, C. P.; Greenleaf, W.; Bennett, L. Analysis of 2-methylisoborneol in water, mud, and channel catfish (*Ictalurus punctatus*) from commerical culture ponds in Mississippi. *Can. J. Fish Aquat. Sci.* **1987**, *44*, 909–912.

McGuire, M. J.; Krasner, S. W.; Hwang, C. J.; Izaguirre, G. Closed-loop stripping analysis as a tool for solving taste and odor problems. *J. Am. Water Works Assoc.* **1981**, *73*, 530–537.

Medsker, L. L.; Jenkins, d.; Thomas, J. F. Odorous compounds in natural waters. An earthy-smelling compound associated with blue-green algae and actinomycetes. *Environ. Sci. Technol.* **1968**, *2*, 461–466.

Oka, K.; Hara, S. Nuclear magnetic resonance spectra of *syn* and *anti* isomers of steroidal 3-ketoximes. *Chem. Ind.* **1968**, 911–912.

Rosen, A. A.; Safferman, R. S.; Mashni, C. I.; Romano, A. H. Identity of odorous substance produced by Streptomyces griseoluteus. *Appl. Microbiol.* **1968**, *16* (1), 178–179.

Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*; Wiley & Sons: New York, 1981; p 272.

Stanker, L. H.; Bigbee, C.; Emon, J. V.; Watkins, B.; Jensen, R. H.; Morris, C.; Vanderlaan, M. An Immunoassay for pyrethoids: Detection of permethrin in meat. *J. Agric. Food Chem.* **1989**, *37*, 834–839.

Sugiura, N.; Yagi, O.; Sudo, K. Musty odour from blue-green alga, *Phormidium tenue*, in Lake Kasumigaura. *Environ. Technol. Lett.* **1986**, *7*, 77–86.

Tabachek, J.; Yurkowski, M. Isolation and identification of blue-green algae producing muddy odour metabolites: geosmin and 2-methylisoborneol in saline lakes in Manitoba. *J. Fish Res. Board Can.* **1976**, *33*, 25–35.

Teshima, R.; Kawase, M.; Tanaka, T.; Hirai, K.; Sato, M.; Sawada, J.; Ikebuchi, H.; Ichinoe, M.; Terao, T. Production and characterization of a specific monoclonal antibody against mycotoxin zearalenone. *J. Agric. Food Chem.* **1990**, *38*, 1618–1622.

Wood, N. F.; Snoeyink, V. L. 2-methylisoborneol, improved synthesis and a quantitative gas chromatographic method for trace concentrations producing odor in water. *J. Chromatogr.* **1977**, *132*, 405–420.

Yagi, S. Musty odour problems in Lake Biwa 1982–1987. *Water Sci. Technol.* **1988**, *20*, 133–142.

Yurkowski, M.; Tabacheck, J. L. Geosmin and 2-methylisoborneol implicated as a cause of muddy odor and flavor in commercial fish from Cedar Lake, Manitoba. *Can. J. Fish Aquat. Sci.* **1980**, *37*, 1449–1450.

Zoretic, P. A.; Branchaud, B.; Maestrone, T. Robinson anneallations with a β-chloroketone in the presence of an acid. *Tetrahedron Lett.* **1975**, *8*, 527–258.

Received for review September 11, 1990. Accepted November 26, 1990.