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**Registry No.** (R)-Citronellyl- $\beta$ -D-glucopyranoside, 117895-55-3; (S)-citronellyl- $\beta$ -D-glucopyranoside, 99096-57-8; (R)-linalyl- $\beta$ -D-glucopyranoside, 99096-59-0; (S)-linalyl- $\beta$ -D-glucopyranoside, 104154-37-2; (S)- $\alpha$ -terpinyl- $\beta$ -D-glucopyranoside, 89616-07-9; (R)- $\alpha$ -terpinyl- $\beta$ -D-glucopyranoside, 114673-99-3; geranyl- $\beta$ -D-glucopyranoside, 22850-13-1; neryl- $\beta$ -D-glucopyranoside, 22850-14-2;  $\beta$ -glucosidase, 9001-22-3.

## A Bromine-Based Color Reaction for the Detection of Geosmin

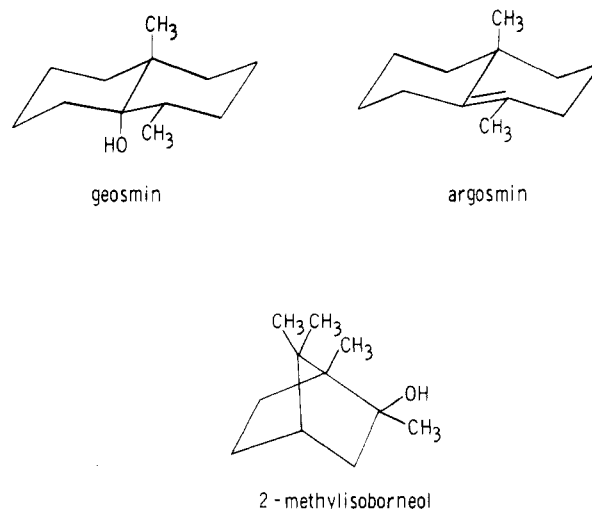
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Geosmin is responsible for natural flavors in beans and beets and for off-flavors in water and fish. This tertiary decalol reacts with bromine in the presence of formic acid to produce a blue complex with maximum absorbance at 650 nm. A method for carrying out this reaction is described, and a possible mechanism is discussed.

Geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) (Figure 1) has been identified as a musty/earthy odor and off-flavor causative compound in water (Rosen et al., 1969; Person, 1981), beets (Murray et al., 1975), beans (Buttery et al., 1975), and fish (Person, 1981; Lovell and Sackey, 1973; Johnsen and Kuan, 1987). The compound is organoleptically detectable in the parts per billion range (Huang et al., 1984). However, instrumental methods for detection of the compound have been limited to gas chromatographic methods. Most of these are modifications of purge and trap methods utilizing various phase separations based on the volatility and/or hydrophobicity of the tertiary decalol (Buttery et al., 1976; Huang et al., 1984; Johnsen and Kuan, 1987). Such methods, though sensitive and accurate, are slower than spectrophotometric or HPLC assays. Such spectrophotometric and HPLC methods for geosmin have not been practical due to the absence of natural or inducible ultraviolet or visible absorptivity of the compound.

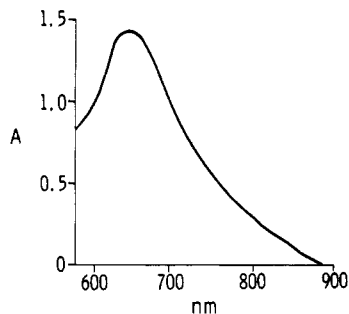
The Tortelli-Jaffe (TJ) reaction is a bromine-based color reaction stated to be specific for compounds that contain either naturally occurring or chemically induced



**Figure 1.** Structure of geosmin, argosmin, and 2-methylisoborneol.

ditertiary double bonds (Tortelli and Jaffe, 1915; Fieser and Fieser, 1959; Hensarling and Jacks, 1983). Since geosmin dehydrates to argosmin (Gerber, 1979), which contains such a ditertiary double bond (Figure 1), it was

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**Figure 2.** Spectrum of the product of the TJ reaction with geosmin. Ordinate is absorbance at wavelength given on abscissa.

of interest to determine whether a practical color reaction for geosmin or argosmin could be developed via the TJ reaction. In this paper we report the reaction of geosmin with bromine by a modified TJ reaction to produce a chromophore with maximum absorptivity at 650 nm.

#### MATERIALS AND METHODS

Geosmin was obtained from Givaudan Corp. Argosmin was prepared by acidification of geosmin, and 2-methylisoborneol (MIB) was obtained from Leon Chance of this laboratory. Stock solutions of geosmin and bromine were prepared in dry dichloromethane (0.5% and 4% (v/v), respectively). Utmost caution should be used in dealing with the reactants. One can be (and has been) burned without realizing it—the burning is painless and blisters appear hours after contact. Optimum results were obtained by the following procedure. The 4% bromine solution was added dropwise to 0.2 mL of the 0.5% geosmin solution with gentle swirling after the addition of each drop. At no time was there an excess of bromine present. After several drops, a faint yellow persisted and the titration was stopped. Five drops of 99% formic acid was added, and a blue developed. The solution was diluted to 3 mL with dichloromethane, and color development was monitored at 700–500 nm at 15-min intervals with a Beckman DU-7 spectrophotometer. The absorbance intensified for 1.5 h and then slowly decreased.

#### RESULTS AND DISCUSSION

The TJ reaction (Tortelli and Jaffe, 1915) as modified according to Bigoni (1956, 1957) and later modified by Hensarling and Jacks (1983) was further modified by reducing sample volume and employing postreaction dilutions to increase sensitivity of detection. At the absorbance maximum of 650 nm (Figure 2) the molar absorptivity was about  $800 \text{ L mol}^{-1} \text{ cm}^{-1}$ . Reference blanks of dichloromethane, the reaction mix without geosmin, the reaction mix without formic acid, and the reaction mix with excess bromine all yielded zero absorbance at 650 nm. The blue did not develop with a large excess of bromine, indicating that care must be taken in achieving the persistent yellow. It was also critical that the proper ratio of bromine to geosmin be present prior to the addition of the formic acid. It is proposed that formation of the chromophore resulted from complexing of a relatively stable intermediate tertiary carbonium ion with persistent bromine. When the reaction was attempted with argosmin prepared in the laboratory by acid treatment of geosmin and with MIB (Figure 1), another compound containing a tertiary hydroxyl causing musty/muddy off-flavors in water and fish (Gerber, 1979; Persson, 1981; Johnsen and Kuan, 1987), the blue did not develop. The lack of reac-

tivity of argosmin was surprising since it contains the required ditertiary double bond. However, as prepared, it probably contained residual acid and thereby did not meet the critical requirement of order of addition of reactants. Since the reaction was successful with geosmin, further attempts with argosmin were not pursued. Although MIB contains a tertiary hydroxyl, it cannot dehydrate to a ditertiary double bond or produce a tertiary carbonium ion due to the absence of the requisite single proton at an adjacent carbon.

We report the participation of geosmin in the TJ reaction as the first known color reaction for geosmin and as the first reported involvement of a tertiary hydroxyl in the TJ reaction. Since the sensitivity of the reaction is less than organoleptic thresholds, we do not propose it as a direct method for determination of geosmin in water or fish. However, combined with present solid-phase and liquid concentration methodology or as a method of post-column derivatization for HPLC, it could be of interest. Other points that could be of future interest are the chemical structure of the chromophore, studies into the order of the reaction, and studies of the reaction with other tertiary hydroxyls.

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