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Geosmin, an Earthy-Smelling Substance Isolated from Actinomycetes

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Summary

Geosmin, an earthy smelling substance, has been obtained from several actinomycetes in addition to those previously reported. On the basis of the NMR and mass spectra of geosmin and its acid transformation product, argosmin, tentative partial structures have been proposed for both.

Introduction

It has been known for a long time that some actinomycetes produce an earthy odor.[1] This odor and taste can become a problem in water supplies,[2] fish,[3] milk, cocoa beans, and foods in storage.[4] One of the groups recently investigating this problem[5] obtained concentrates which could be diluted one billion fold and still retain their characteristic odor.[6] Our work showed that one specific compound having an earthy odor can be found among the metabolities of many actinomycetes.[7] We have named this substance geosmin and an exchange of samples with Dr. Romano[6] showed that geosmin is identical with the odor active component of his concentrates.

Production of Geosmin

Until recently, the highest production of geosmin, 1 mg./liter of whole broth, was from *Streptomyces griseus* LP-16.[7] The results of our latest survey are summarized in Table I. Whole broths were distilled and the distillate extracted with methylene chloride. Geosmin was isolated in pure form from concentrated methylene chloride extracts by preparative gas chromatography. It is a colorless neutral oil which darkens very slightly on long storage even at 5°C. in a sealed tube. It soon became apparent that geosmin was not stable to acid but was transformed readily to another colorless oil of slightly lower

TABLE I
Geosmin Production by Various Actinomycetes as Detected by Gas Chromatography

Organism	Production medium,[b] 250 ml./2-l. flask	Day of maximum production	Yield of geosmin,[a] μg./ml.
Streptomyces sp. 27-20	YD	14	0.36
" " B-5a	SBM/J	7	0.38
" " B-7	"	4	1.0
" " 37-12	"	7	0.24
S. alboniger 12462	"	9	0.23
S. lavendulae 3440 1-Y	YD	5	0.46
S. viridochromogenus 94	M-5	8	3.9
Nocardia sp. SS1/1	SBM	5	0.24
" " W-68	SBM/J	7	0.36
" " I-15	YD	8	1.6
Microbispora rosea 3748	Pablum	12	5.8

[a] Quantitative gas chromatographic determinations: 1 μg. of pure geosmin = a peak with an area of 8 mm.^{2} under the conditions used.

[b] SBM/J = 10 g. soybean meal, 10 g. Wilson's peptone No. 851 (The Wilson Co., Chicago, Ill.), 20 g. Cerelose, 5 g. sodium chloride, tap water to 1 liter, adjust pH to 7.5. M-5 = 5 g. BYF 50-X (a fraction of autolysed brewers yeast sold by Amber Laboratories, Inc., Milwaukee, Wis.), 5 g. Wilson's peptone No. 851, 10 g. Cerelose, 20 g. Brer Rabbit Green Label Molasses, tap water to 1 liter, adjust pH to 8.5. For other media see ref. 7.

retention time on the gas chromatograph and of absolutely no odor, which we called argosmin.[7]

Properties of the Osmins

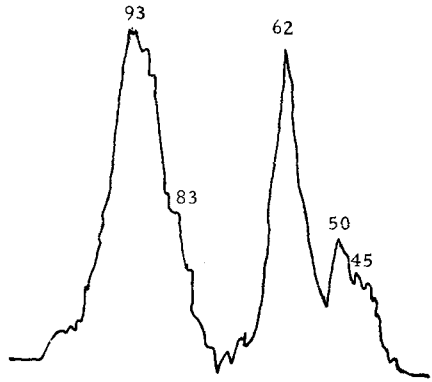
By gas chromatography we were able to prepare about 15 mg. of each oil in pure form. Their properties are summarized in Table II.

TABLE II
Physical Properties of the Osmins

	B.p., °C	$[\alpha]_D^{25}$	$E_{1cm}^{1\%}$ in MeOH		
			220, mμ	230, mμ	240 mμ
Geosmin	270	−16.5	8.6	7.3	6
Argosmin	230	+29	56	17	8

The boiling points were determined by comparing their retention times with those of a series of nonpolar compounds of known boiling point under identical isothermal conditions. The infrared spectra indicated the absence of O—H, N—H, or carbonyl groups.

High resolution mass spectroscopy indicated molecular formulas of $C_{12}H_{22}O$ for geosmin and $C_{12}H_{20}$ for argosmin. From the spectra we could deduce that loss of methyl and loss of C_4H_8 were important processes in both molecules. The fragments from geosmin could be divided into two groups; those containing oxygen and those from the molecule formed by loss of water. The fragments from the latter were similar to those from argosmin which argues for a similar carbon skeleton for both. The most common fragmentation for monoterpene hydrocarbons and alcohols is loss of methyl and C_3H_7,[8] the latter



Group	cps	δ	Area by weight, mg.	Best fit for 21 H
C—CH_2—C and —CH	93	1.55	32	13
	83 (sh)	1.43		
CH_3—C—	62	1.03	21	9 = 3 CH_3
	50	0.83		
	45 (sh)	0.75		

Fig. 1. NMR spectrum of Geosmin.

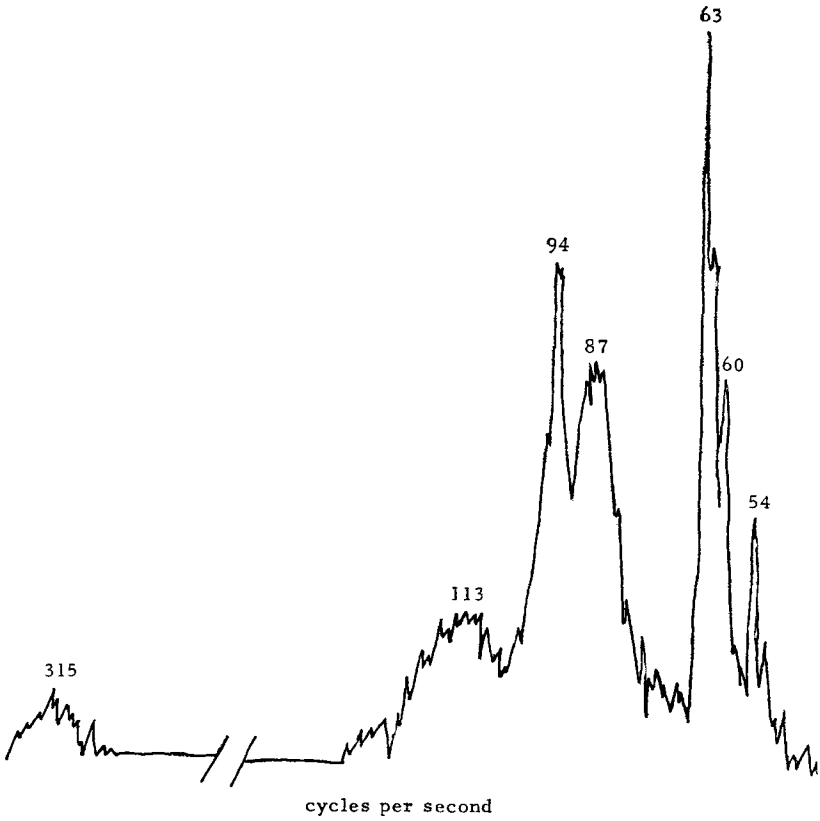


Fig. 2. See caption, p. 325.

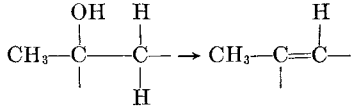
an isopropyl group from a *gem* dimethyl structure. The relative unimportance of the C_3H_7 fragment in our spectra suggests the absence of any *gem* dimethyl structure.

The NMR curves are shown in Figures 1 and 2. Spectra were taken on 3.5% solutions in carbon tetrachloride using a Varian A-60, micro cells, and tetramethyl silane as internal standard. The 22nd hydrogen in geosmin is in the OH group. It is difficult to assign a precise location for hydrogens on oxygen; their bands are frequently broad and thus escape detection. The decrease in the number of hydrogens for methyl on saturated carbon in the transformation of

Group	cps	δ	Area by weight, mg.	Best fit for 21H
—CH=C<	315	5.25	~5	1
CH_3—C=C	113	1.92	24	3 = 1 CH_3
C—CH_2—C and	94	1.58	71	10
—CH	87	1.45		
CH_3—C—	63	1.05	39	6 = 2 CH_3
	54, 57, 60	0.95		

Fig. 2. NMR spectrum of argosmin.

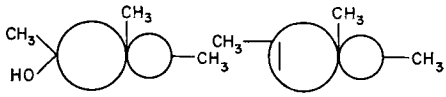
geosmiun to argosmiun and the appearance of the 113 and 315 cps peaks suggest this kind of reaction:



The ultraviolet absorbtion of pure geosmiun in methanol at 210 mμ had an intensity $\epsilon = 263$. This low value excludes any tetrasubstituted double bonds which would be undisclosed by NMR since molecules with tetrasubstituted double bonds have ϵ values at 210 mμ of 3400–4700.[9] Thus since geosmin has no double bonds it must have two rings.

Discussion

With the evidence given it is possible to write tentative partial structures for the osmins which take into account all but the lack of O—H absorbtion in the infrared for geosmin. Structures for geosmin



in which the oxygen is in some kind of cyclic ether run into difficulty because acid-catalyzed ring opening and loss of water would give $C_{12}H_{22}$ for argosmin. To obtain $C_{12}H_{20}$ it becomes necessary to postulate a simultaneous ring closure and loss of two hydrogens or formation of a second double bond which is nonconjugated (because there is no conjugated diene maximum in the ultraviolet) and completely substituted (because the NMR shows only one olefinic proton). This is highly unlikely in view of the similarity of the fragmentation patterns of the two molecules in the mass spectrometer. The angular methyl groups are indicated by the important peaks in the mass spectra showing loss of methyl, and the unsplit methyl peaks at 63 cps in the NMR spectra.

Recently, another group has reported the isolation, identification, and discussed partial structures for a musty smelling and tasting substance from actinomycetes.[10] Their strains were isolated from the Cedar River in Iowa during an episode of mustiness in 1961 which was extremely resistant to treatment. The molecular formula of their substance is $C_{12}H_{18}O_2$ and the properties are quite different from geosmin. They report ultraviolet absorbtion at 225 and 301 mμ, carbonyl absorbtion in the infrared at 5.84 mμ, and clear NMR evidence for an isopropyl group. This brings to mind the stereochemical theory of olfaction[11] which states that the odor of a compound is determined by the size and shape of its molecule. Thus there may well be numerous musty or earthy-smelling substances which will undoubtedly have different structures. However, it appears quite possible that they will have a similar size and shape. Furthermore, if one can get any good idea about the size and shape of earthy-smelling molecules, it may aid in the final decisions about the stereochemistry of geosmin.

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