A solution of 2.0 g. of the acid from the last hydrolysis in 10 ml. of methanol was saturated with dry hydrogen chloride. The mixture was allowed to stand in the laboratory for 48 hr. Upon concentrating the reaction mixture followed by the addition of a small amount of water, shiny white crystals, m.p. 68.5°, were obtained.

Anal. Calcd. for $C_{14}H_{22}O_8$: C, 52.82; H, 6.97. Found: C, 52.60; H, 6.91.

A mixture melting point of the acid, m.p. 215–216°, with authentic hexane-1,3,4,6-tetracarboxylic acid and of the methyl esters from each showed no depressions.

Hexane-1,3,4,6-tetracarboxylic Acid (XIII).—The acid, m.p. 215°, was synthesized according to the direction of Ruzicka and co-workers[35] by the hydrolysis of the cyano ester resulting from the reaction of ethyl α-cyanoglutarate (XI) and ethyl α-bromoglutarate (XII) in the presence of sodium ethoxide. Esterification of the acid with methanol, as described above, gave the methyl ester, m.p. 68.5°.

(35) L. Ruzicka, A. Borges de Almeida, and A. Brack, *Helv. Chim. Acta*, **17**, 183 (1934).

Acknowledgment.—We are indebted to Professor A. H. Corwin for his initial proposal of a mechanism from which ours is derived, to Dr. R. F. Curtis for his assistance with early phases of the investigation, and to R. W. Richardson for the discussion concerning X-ray results and symmetry. We are also grateful to Dr. J. H. Wotiz for kindly communicating the results of his independent study, in collaboration with Dr. D. E. Mancuso, concerned with the nature of the hexadiene octaester, as well as to Dr. J. Ogilvie and the Microanalytical Department of the Wyeth Laboratories, Inc., for molecular weight determinations.

The Stereoisomeric Farnesols[1]

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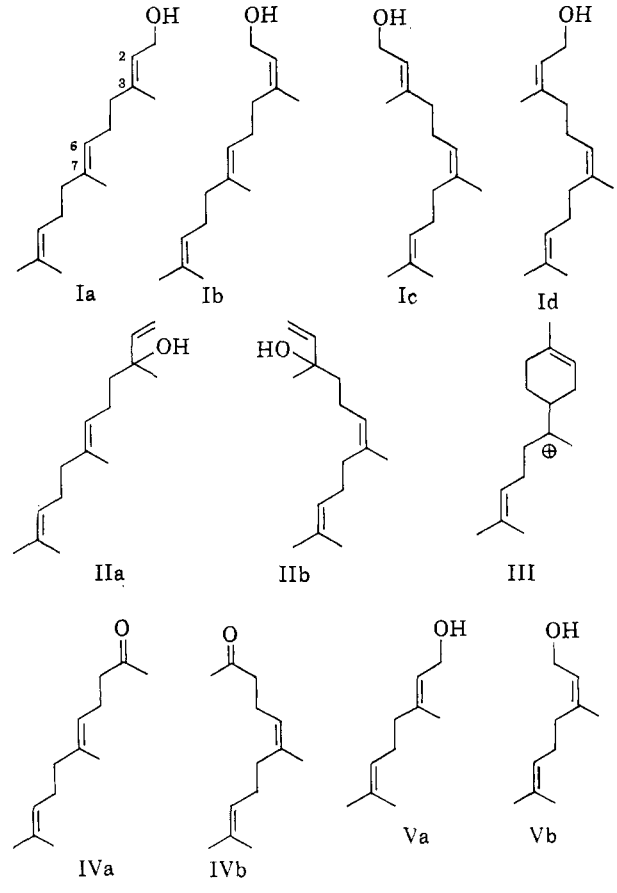
The preparation and characterization of the four stereoisomeric farnesols (Ia–d) is described. Farnesol from *Hibiscus abelmoschus* and farnesic acid prepared *via* the S-benzylthiuronium salt, m.p. 130–131°, were shown to be the *trans–trans* isomers.

Because of its pleasing odor, farnesol (I) has long been used in perfumery.[2] Although it has been reported to occur in many plants, it is always a minor constituent, and is prepared commercially by isomerizing the much more abundant natural substance *trans*-nerolidol (IIa).[3] Farnesol has recently taken on new importance, since it has become increasingly clear that a farnesol derivative (or derivatives) is a key intermediate in the biosynthesis of many substances: almost all other sesquiterpenoids, almost all diterpenoids and triterpenoids, and all steroids and carotenoids.[4] In particular, farnesyl pyrophosphate has been demonstrated to be a biological precursor of squalene,[5] which is then converted into other triterpenoids and into steroids. It has been reported recently that farnesol and farnesal exhibit juvenile hormone action in insects.[6]

Although farnesol was first isolated over fifty years ago, was (appropriately, in view of the position of its esters in biogenetic schemes) the first sesquiterpenoid of known constitution, and has been synthesized many times,[3,5b,7] little is known regarding its stereochemistry. The stereochemistry of farnesol derivatives is undoubtedly of biological importance, since only a *trans-trans* derivative can give squalene, whereas a *cis–trans*[8] or *cis–cis* derivative is needed to give bisabolyl carbonium ion (III), a probable intermediate in the formation of many sesquiterpenoids.[4]



From petitgrain oil, Naves[9] obtained an alcohol which on chromic acid oxidation gave a farnesal whose semicarbazone (m.p. 127.5–128°) melted lower than

(1) Terpenoids, Part VII.

(2) E. Guenther, "The Essential Oils," Vol. II, D. Van Nostrand Company, Inc., New York, N. Y., 1949, p. 258.

(3) *e.g.*, L. Ruzicka and G. Firmenich, *Helv. Chim. Acta*, **22**, 392 (1939).

(4) L. Ruzicka, A. Eschenmoser, and H. Heusser, *Experientia*, **9**, 362 (1953); P. Crabbe and G. Ourisson, *Ind. chim. Belge*, **22**, 1309 (1957); J. B. Hendrickson, *Tetrahedron*, **7**, 82 (1959).

(5) (a) F. Lynen, H. Eggerer, U. Henning, and I. Kessel, *Angew. Chem.*, **70**, 738 (1958); (b) G. Popjak, J. W. Cornforth, R. H. Cornforth, R. Ryhage, and D. S. Goodman, *J. Biol. Chem.*, **237**, 56 (1962).

(6) P. Schmialek, *Z. Naturforsch.*, **16b**, 461 (1961).

(7) *e.g.*, (a) L. Ruzicka, *Helv. Chim. Acta*, **6**, 492 (1923); (b) I. N. Nazarov, B. P. Gusev, and V. I. Gunar, *Zh. Obshch. Khim.*, **28**, 1444 (1958); (c) E. Y. Shvarts and A. A. Petrov, *ibid.*, **30**, 3598 (1960).

(8) The first configurational designation refers to the 2,3 double bond, the second to the 6,7 double bond.

(9) Y.-R. Naves, *Helv. Chim. Acta*, **32**, 1798 (1949).

farnesal semicarbazone from other sources (m.p.'s ranging from 132–133° to 138–139°). The farnesals with the highest and lowest melting semicarbazones were both found to give geranylacetone (IVa) on degradation, and it was then assumed that the former was the *cis–trans* isomer and the latter the *trans–trans* isomer. Later,[10] vapor phase chromatography was employed with farnesol mixtures: Using a commercial silicone on firebrick column,[11] farnesol from the isomerization of *trans*-nerolidol (IIa) gave only one peak, whereas farnesol from the isomerization of a mixture of nerolidols (IIa and b) gave three peaks. It was assumed that the former farnesol was the *trans–trans* isomer (Ia), that the *cis–trans* isomer (Ib) was decomposing under the vapor phase chromatography conditions, and that the three farnesol peaks were due to the *trans–trans*, *trans–cis*, and *cis–cis* isomers (Ia, c, and d). The *trans–trans* isomer is stated to be the most rapidly eluted one. More recently,[12] Naves has stated that *trans–trans*-farnesol (Ia) is the only stereoisomer present in many essential oils but that it occurs mixed with the *cis–trans* isomer (Ib) in petitgrain oil and several other oils; *trans–trans* stereochemistry was assigned to the stereoisomer with the longer vapor phase chromatography retention time, by analogy with geraniol (Va) and nerol (Vb). Popjak and Cornforth[13] have reported analyzing commercial farnesol (from Firmenich, Inc., Geneva) by vapor phase chromatography and finding three peaks, the first of which coincided with the peak from *trans*-nerolidol (IIa); the other two peaks were presumed to be due to *trans–trans*- and *cis–trans*-farnesols (Ia and b), the assignments again being based on a longer retention time for the former stereoisomer. One farnesol synthesis[7b] has been reported to give only the natural isomer, whose configuration was not mentioned. Another synthesis[5b] was found to give approximately equal amounts of isomers differing in configuration at the 2,3 double bond.

In only one of the above cases describing the analysis of farnesol mixtures by vapor phase chromatography was the collection of a farnesol reported: In that case[5b] the isomer assigned the *trans–trans* configuration was collected and its infrared spectrum taken. Thus, prior to the present study, it appeared that only one of the four farnesols, assigned the *trans–trans* configuration on questionable grounds, had been obtained in pure form.

The goal of the present study was to find ways of obtaining and characterizing all four stereoisomeric farnesols, and to establish unequivocally the configuration of natural farnesol.

Results

Commercial farnesol[14] was separated into the *trans–trans* (Ia; 55-75%) and *cis–trans* (Ib; 25–35%) isomers by vapor phase chromatography using several different column packings. In early experiments it was found that farnesols were decomposed on a variety of commercial columns with firebrick packings, including three columns of the type[11] employed by Naves and Odermatt.[10] It was found essential to employ base-washed firebrick to avoid this decomposition.

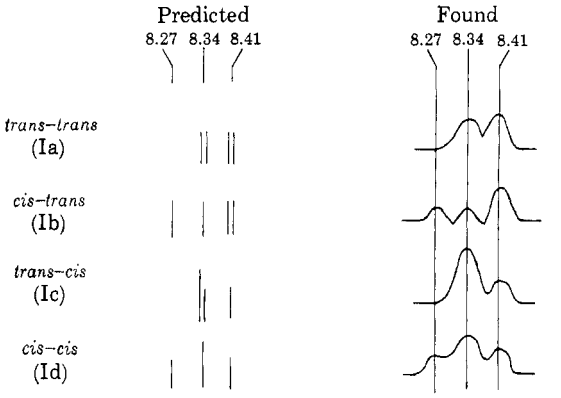


Fig. 1.—Methyl proton regions of farnesol n.m.r. spectra. Chemical shifts are in τ units.

Fractional distillation, a good method for separating geraniol–nerol (Va–Vb) mixtures,[15] was relatively ineffective for the separation of these two farnesols.[16]

Pure *trans–trans*-farnesol (Ia) was also obtained from the commercial mixture by preparing the diphenylurethane and recrystallizing to constant m.p. (61–63°; Naves[17] reported m.p. 54.5–55° for a farnesyl diphenylurethane of unknown stereochemistry). Saponification gave a mixture of *trans–trans*-farnesol (Ia) and diphenylamine, which was easily separated by preparative vapor phase chromatography or by chromatography on silica gel. The over-all yield was 19%.

Isomerization[3] of *cis*-nerolidol[18] (IIb) gave, in 50% yield, a 3:1 mixture of *trans–cis*- and *cis–cis*-farnesols (Ic and d). This mixture was separated by preparative vapor phase chromatography.

S-Benzylthiuronium farnesate, m.p. 130–131°, was found to be the *trans–trans* isomer by lithium aluminum hydride reduction to stereochemically pure *trans–trans*-farnesol (Ia), contaminated with some *trans*-2,3-dihydrofarnesol.

Farnesol from ambrette seeds (*Hibiscus abelmoschus*) was shown to be the *trans–trans* isomer (Ia) by comparison with the synthetic farnesols.

Some properties of the pure farnesols (Ia–d) are listed in Table I; portions of their n.m.r. spectra are reproduced in Fig. 1.

Discussion

The method of formation, elemental analyses, nearly identical infrared spectra, and particularly the n.m.r. spectra leave no doubt that the compounds described above as farnesols all have the farnesol constitution. The stereochemical assignments can be made in the three following ways.

(1) The isomerization of *trans*-nerolidol (IIa) by the usual procedure[3] (*trans*-nerolidol (IIa) $\xrightarrow{PBr_3}$ bromide $\xrightarrow{OAc^{\ominus}}$ acetate $\xrightarrow{OH^{\ominus}}$ farnesol + nerolidol) should give a mixture of *trans–trans*- and *cis–trans*-farnesols (Ia and b) with the former predominating, and *cis*-ner-

(10) Y.-R. Naves and A. Odermatt, *Bull. soc. chim. France*, 377 (1958).
(11) Column "C" of the Perkin–Elmer Corp., Norwalk, Conn.
(12) Y.-R. Naves, *Compt. Rend.*, **251**, 900 (1960).
(13) G. Popjak and R. H. Cornforth, *J. Chromatog.*, **4**, 214 (1960).
(14) From Firmenich, Inc., Geneva; Fluka AG, Buchs SG; and L. Light and Co. Ltd., Colnbrook, Bucks, England.
(15) P. Nicholas, B.S. thesis, University of Illinois, 1960; K. W. Greenlee and V. G. Wiley, *J. Org. Chem.*, **27**, 2304 (1962).
(16) We are indebted to Mr. J. H. Schauble for this result.
(17) Y.-R. Naves, *Helv. Chim. Acta*, **29**, 1084 (1946).
(18) A. Ofner, W. Kimel, A. Holmgren, and F. Forrester, *ibid.*, **42**, 2577 (1959).

TABLE I
PROPERTIES OF FARNESOL ISOMERS

Isomer	$n^{25}D$	Vapor phase chromatography retention time, min.[a]	Found % C[b]	Found % H[b]
trans–trans (Ia)	1.4872	29	80.79	11.64
cis–trans (Ib)	1.4865	26	80.64	11.72
trans–cis (Ic)	1.4869	26	80.76	11.77
cis–cis (Id)	1.4861	22	80.54	11.89

[a] 5-ft. column containing 20% Carbowax 20-M on base-washed firebrick at 210°. [b] Calcd. for $C_{15}H_{26}O$: C, 81.02; H, 11.78.

olidol (IIb) should give mainly the *trans–cis* isomer (Ic) accompanied by some of the *cis–cis* isomer (Id). It is not clear whether the stereochemistry of the 2,3 double bond is established when the bromides are equilibrated, or in a rate-controlled process as the bromides react with acetate ion, but in either case steric effects should favor the formation of the stereoisomer with the *trans* 2,3 double bond. Thus, the stereoisomer favored in synthesis (by between 1.5:1 and 3:1) was in each case assigned a *trans* 2,3 double bond, and the other isomer a *cis* 2,3 double bond.

This argument assumes that the configuration of the 6,7 double bond was unaffected during the isomerization sequence, an assumption justified by the finding that each nerolidol isomer gave two unique farnesols. The argument also requires that the configurations of the nerolidols have been correctly assigned.[18] These configurations were assigned by synthesis from geranylacetone (IVa) and nerylacetone (IVb), whose stereochemistry rests largely on the argument that the isomer with the higher boiling point and refractive index should be the *trans* isomer. We have corroborated these assignments by examination of the n.m.r. spectra of geranylacetone (IVa), nerylacetone (IVb), and the nerolidols (IIa and b),[19] and by stereospecifically synthesizing geranylacetone (IVa) from geraniol (Va) and nerylacetone (IVb) from nerol (Vb).[20] The configurations of geraniol (Va) and nerol (Vb) are well established.[21]

(2) By analogy with geraniol (Va) and nerol (Vb), *trans–trans*-farnesol (Ia) would be expected to have the highest refractive index, highest boiling point, and longest retention time on a Carbowax column of any of the stereoisomers; *cis–cis*-farnesol (Id) should be at the other end of the scale in these properties; the other two isomers (Ib and c) should fall in between. As can be seen from Table I, the refractive indices and retention times support the assignments made above for Ia and d on the basis of method of synthesis. In addition, the partial separation of the farnesols in commercial farnesol achieved by fractional distillation indicated that the isomer assigned the *cis–trans* configuration boils lower than the isomer assigned the *trans–trans* configuration.[16]

(19) R. B. Bates, R. H. Carnighan, R. C. Rakutis, and J. H. Schauble, *Chem. Ind.* (London), 1020 (1962).

(20) R. B. Bates, D. M. Gale, B. J. Gruner, and P. P. Nicholas, *ibid.*, 1907 (1961); experimental details of these and related reactions are forthcoming.

(21) J. W. K. Burrell, L. M. Jackman, and B. C. L. Weedon, *Proc. Chem. Soc.*, 263 (1959).

(3) The n.m.r. spectra of the farnesols (Fig. 1) confirm the above stereochemical assignments. Some of the methyl groups in each isomer absorb at the same location, but fortunately the predicted patterns[22] (Fig. 1) are different for each isomer.

In their classic study of the stereochemistry of polyene cyclization, Stork and Burgstahler[23] made the assumption that farnesic acid regenerated from S-benzylthiuronium farnesate, m.p. 132–133°, is the pure *trans–trans* isomer. Although this assumption was vital to some of their arguments, the basis on which it was made was not stated. Our reduction of the thiuronium salt with lithium aluminum hydride to stereochemically pure *trans–trans*-farnesol (Ia), coupled with the finding that similar reduction of a mixture (m.p. 115–118°) of S-benzylthiuronium *trans–trans*- and *cis–trans*-farnesates gives a mixture of *trans–trans*- and *cis–trans*-farnesols (Ia and b), shows that their assumption was correct.

Experimental

Melting points were taken in capillary tubes and are corrected.

N.m.r. spectra were measured at 60 Mc. on Varian HR-60 and A-60 instruments, using 10% solutions in carbon tetrachloride and internal tetramethylsilane and cyclohexane standards.[19]

In all cases where farnesols are reported below as reaction products, the reaction mixture was analyzed by vapor phase chromatography with characterization of collected samples of the farnesols *via* infrared and n.m.r. spectra. Of the many column packings tried, the following were the most satisfactory: the Aerograph detergent and G. E. SF-96 silicone on Fluoropak columns (Wilkens Instrument and Research, Walnut Creek, Calif.), and Carbowax 20-M on either Fluoropak or base-washed firebrick. The base-washing, essential with firebrick to avoid decomposition of the farnesols, was done with 5 *N* potassium hydroxide in methanol, and was followed by washing with methanol to pH 8. The retention times reported in Table I and below refer to a 5-ft. column of 0.5 in. diameter packed with 20% Carbowax 20-M on 30:60 base-washed firebrick at 210°, and a helium flow rate of 60 ml./min. A large-scale apparatus with this packing was sometimes employed; this had four parallel 10-ft. 0.75-in. columns and took 4-ml. samples of farnesol mixtures.

Separation of *trans–trans*-Farnesol (Ia) from *cis–trans*-Farnesol (Ib).—Using the 5-ft. Carbowax column described above, 5–50 μl. samples of commercial farnesol [2% *trans*-nerolidol (IIa; retention time 10 min.), 27% *cis-trans*-farnesol (Ib), and 71% *trans–trans*-farnesol (Ia)] yielded 100 mg. of Ia and 50 mg. of Ib, both of 99 + % purity. Properties of these samples are recorded in Table I and Fig. 1.

Using the large-scale vapor phase chromatography apparatus described above and collecting the first half of the peak for Ib and the last half of the Ia peak, 4 ml. of this commercial farnesol gave 0.9 g. of Ia (91% pure) and 0.3 g. of Ib (81% pure).

A 20-ml. sample of this commercial farnesol was distilled through a 14-in. Podbielniak column at 0.35 mm. (b.p. 111°). The first milliliter of distillate was 6% IIa, 36% Ib, and 58% Ia; the last milliliter was 23% Ib and 77% Ia.[16]

***trans–trans*-Farnesyldiphenylurethane.**—A mixture of 22.2 g. (0.1 mole) of commercial farnesol, 23.2 g. (0.1 mole) of diphenylcarbamyl chloride, and 24 ml. of pyridine was heated at 100–105° under nitrogen for 4 hr. The brown reaction mixture was taken up in 150 ml. of ether, and the ether solution was washed in turn with tartaric acid solution until acidic, sodium bicarbonate solution until basic, and saturated salt solution until neutral. Evaporation of the solvent yielded a dark semisolid, which on five recrystallizations from methanol gave 5.9 g. (20% based on *trans–trans*-farnesol present in the commercial farnesol used) of pure *trans–trans*-farnesyldiphenylurethane, m.p. 61–63°.

Anal. Calcd. for $C_{28}H_{35}O_2N$: C, 80.53; H, 8.45; N, 3.36. Found: C, 80.79; H, 8.63; N, 3.54.

(22) R. B. Bates and D. M. Gale, *J. Am. Chem. Soc.*, **82**, 5749 (1960).

(23) G. Stork and A. W. Burgstahler, *ibid.*, **77**, 5068 (1955).

trans-trans-**Farnesol (Ia) from Its Diphenylurethane.**—A mixture of 10 g. of the derivative, 5.3 g. of potassium hydroxide, 53 ml. of absolute ethanol, and 1 ml. of water was refluxed under nitrogen for 5 hr. and then allowed to stand overnight. The reaction mixture was extracted with ether, and the ether solution was washed until neutral with saturated salt solution, dried, and the ether evaporated, leaving 9.3 g. (93%) of a 1:1 mixture of Ia and diphenylamine (analyzed by vapor phase chromatography).

A portion of this mixture was separated by vapor phase chromatography. Half of the mixture was chromatographed on 75 g. of silica gel. The column was prepared with petroleum ether–benzene; elution with 500 ml. of 10% methanol–90% benzene gave 1.9 g. of Ia, 90% pure by vapor phase chromatography, with the contaminants having retention times suggestive of farnesol dehydration products; none of the other farnesols were detected.

cis-**Nerolidol (IIb).**[18]—Vinylmagnesium bromide was prepared by the dropwise addition of a chilled solution of 33 ml. of vinyl bromide in 60 ml. of tetrahydrofuran to a mixture of 11.3 g. of magnesium, 100 ml. of tetrahydrofuran, and a crystal of iodine at 50–60° with stirring under nitrogen. The mixture was cooled to room temperature and kept at room temperature while a solution of 30 g. of nerylacetone[24] (IVb) containing 3% of geranylacetone (IVa) in 60 ml. of tetrahydrofuran was added dropwise. After stirring overnight, the reaction mixture was poured into saturated ammonium chloride solution, the layers were separated, and the aqueous layer was extracted twice with ether. The combined organic layers were washed with saturated sodium bicarbonate solution and dried over magnesium sulfate. Most of the solvent was removed at room temperature using a water aspirator, and the residue was vacuum distilled to give 20.5 g. (60%) of *cis*-nerolidol, (IIb; retention time 8.5 min.), b.p. 102–104° (0.6 mm.), $n^{25}D$ 1.4759, shown to contain 3% *trans*-nerolidol (IIa) by vapor phase chromatography.

trans-cis- **and** *cis-cis*-**Farnesols (Ic and d).**—To a solution of 5.00 g. of *cis*-nerolidol in 0.5 g. of pyridine at −15°, 2.50 g. of phosphorus tribromide was added dropwise. After stirring at this temperature for 1 hr. and then at room temperature overnight, the reaction mixture was poured into ice-cold saturated potassium carbonate solution and extracted with petroleum ether. The petroleum ether solution of bromides was washed with ice-cold solutions of dilute sulfuric acid, sodium bisulfite, and water, and dried over potassium carbonate. Most of the solvent was removed at room temperature with a water aspirator.

The residual bromide mixture was shaken for 3 days with 6 g. of oven-dried powdered potassium acetate and 80 ml. of dry acetone. The salts were filtered off and washed once with dry acetone, and most of the acetone was evaporated. Pentane was added, the insoluble material was removed by filtration, and the pentane was evaporated.

The residual farnesyl acetate was saponified by refluxing for 3 hr. with 80 ml. of 5% potassium hydroxide in methanol. The resulting solution was cooled and ether and saturated potassium carbonate were added. The ether layer was washed twice with saturated sodium chloride solution and dried over magnesium sulfate. Methanol and ether were evaporated with a water aspirator, and ether and magnesium sulfate were added. After filtration and evaporation of the ether, the residual oil was evaporatively distilled twice at 125° (0.2 mm.), yielding 2.59 g. (52%) of a 3:1 mixture (by vapor phase chromatography) of *trans-cis*- and *cis-cis*-farnesols (Ic and d). These farnesols were separated by preparative vapor phase chromatography; some of the properties of the purest samples (99 + %) are recorded in Table I and Fig. 1.

trans-trans-**Farnesol (Ia) from Ambrette Seeds.**—Over a period of several days, 1 kg. of ground ambrette seeds (Meer Corp., New York) was extracted several times with a total of 4 l. of ether. The ether was evaporated and the viscous oil saponified by refluxing with 20 g. of sodium hydroxide, 100 ml. of water, and 100 ml. of methanol for 1 hr. After extracting with ether, washing with water, and drying over magnesium sulfate, the ether was evaporated and the residue distilled at 1 mm. (pot temp. to 250°). This distillate (about 500 mg.) was shown by vapor phase chromatography, n.m.r., and infrared to be *ca.* 90% pure Ia, containing at most a few percent of Ib.

trans-trans-**Farnesol (Ia) from S-Benzylthiuronium** *trans-trans*-**Farnesate.**—To a mixture of 180 mg. of S-benzylthiuronium *trans-trans*-farnesate, m.p. 130–131°,[23] and 10 ml. of ether was added a suspension of 200 mg. of lithium aluminum hydride in 10 ml. of ether. After stirring at room temperature for 16 hr., 0.2 ml. of water, 0.2 ml. of 15% sodium hydroxide solution, and 0.6 ml. of water were added. Filtration and evaporation of the ether gave 118 mg. of colorless oil, shown by vapor phase chromatography to contain *trans-trans*-farnesol as the only farnesol isomer. There was an earlier peak (retention time, 20 min.; area, one fifth that of the farnesol peak), which was found to be due to *trans*-2,3-dihydrofarnesol (retention time 20 min.). This compound was collected and evaporatively distilled at 140° (2 mm.); its infrared spectrum contained a strong hydroxyl band at 3500 $cm.^{-1}$.

Anal. Calcd. for $C_{15}H_{28}O$: C, 80.29; H, 12.58. Found: C, 80.46; H, 12.31.

Similar reduction of 180 mg. of a mixture (m.p. 115–118°) of S-benzylthiuronium *trans-trans*- and *cis-trans*-farnesates (prepared from a 3:1 mixture of *trans-trans*- and *cis-trans*-farnesols) gave 130 mg. of a mixture with a 4:1 ratio of farnesols to *trans*-2,3-dihydrofarnesol; in this case two farnesols were present, *trans-trans* (Ia) and *cis-trans* (Ib), in a 4:1 ratio.

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(24) P. A. Stadler, A. Nechvatal, A. J. Frey, and A. Eschenmoser, *Helv. Chim. Acta*, **40**, 1373 (1957).