Isolation of geranyl geraniol from the unsaponifiable fraction of linseed oil

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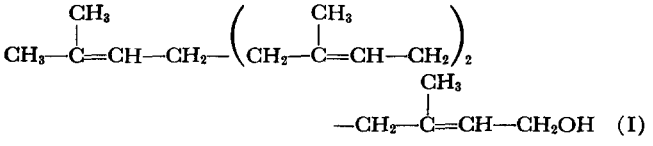
ABSTRACT From the unsaponifiable fraction (63 g) of linseed oil (25 kg), two terpenic alcohols were isolated by alumina column, thin-layer, and gas–liquid chromatography. They were identified as phytol and geranyl geraniol (a precursor of bi- and tricyclic diterpenes) by infrared and nuclear magnetic resonance spectroscopy, ozonolysis, and mass spectrometry.

KEY WORDS linseed oil · unsaponifiable fraction · phytol · geranyl geraniol · isolation · identification · nuclear magnetic resonance · peanut oil

GERANYL GERANIOL (I) has been proposed by some authors as an intermediate in the biosynthesis of di- and tricyclic diterpenes.



(I)

According to Wagner and Jaureg (1) and Kuhn and Grundmann (2) it would be transformed into phytol by hydrogenation and into lycopene and other carotenoids by dehydration and dimerization. Ruzicka suggested (3) that geranyl geraniol and geranyl linalool might be the precursors of the di- and tricyclic diterpenes respectively; but although geranyl linalool has been isolated, together with phytol and isophytol, from jasmine essential oil by Demole and Lederer (4), these authors were unable to isolate geranyl geraniol from the same source, probably because it is rapidly metabolized.

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; NMR, nuclear magnetic resonance; DNP, dinitrophenylhydrazone.

According to some recent work of Costes (5), geranyl geraniol and geranyl linalool are precursors of carotene and lutein. Stobbe (6) has proposed geranyl geraniol as an intermediate in the biosynthesis of vitamin K_2.

Popjak (7) has found, by gas–liquid chromatography of the unsaponifiable fraction of liver homogenate incubated with DL-mevalonate-2-^{14}C, two peaks with the retention volumes expected for geranyl linalool and geranyl geraniol. These were minor constituents of a mixture consisting predominantly of nerolidol, all-*trans* farnesol, methyl allyl alcohol, linalool, nerol, and geraniol.

Geranyl geraniol has, to our knowledge, never been isolated from natural sources and fully characterized although it has been shown to be synthesized by cell-free enzyme systems (8, 9). This article describes the isolation and identification of geranyl geraniol from the unsaponifiable fraction of linseed oil. We have also obtained it from the corresponding fraction of peanut oil.

MATERIAL AND METHODS

For the separation of the unsaponifiable fraction, an Italian linseed oil was utilized. For comparison, samples of phytol and farnesol were purchased from British Drug Houses (Poole, England) and Fluka A.G. (Basel, Switzerland).

Column Chromatography. Alumina (Activity III) in petroleum ether (bp 60–80°C) was used to fractionate the unsaponifiable fraction, and silicic acid (Mallinckrodt Chemical Company, St. Louis, Mo.) for the separation of phytol from geranyl geraniol. Details are given in Results. After separation, each fraction was recovered and weighed, and its composition was checked by TLC.

For *thin-layer chromatography*, 20 × 20 cm plates, 0.25 mm thick, prepared with Silica Gel G (Merck & Co., Inc.) were used. Substances were detected by charring

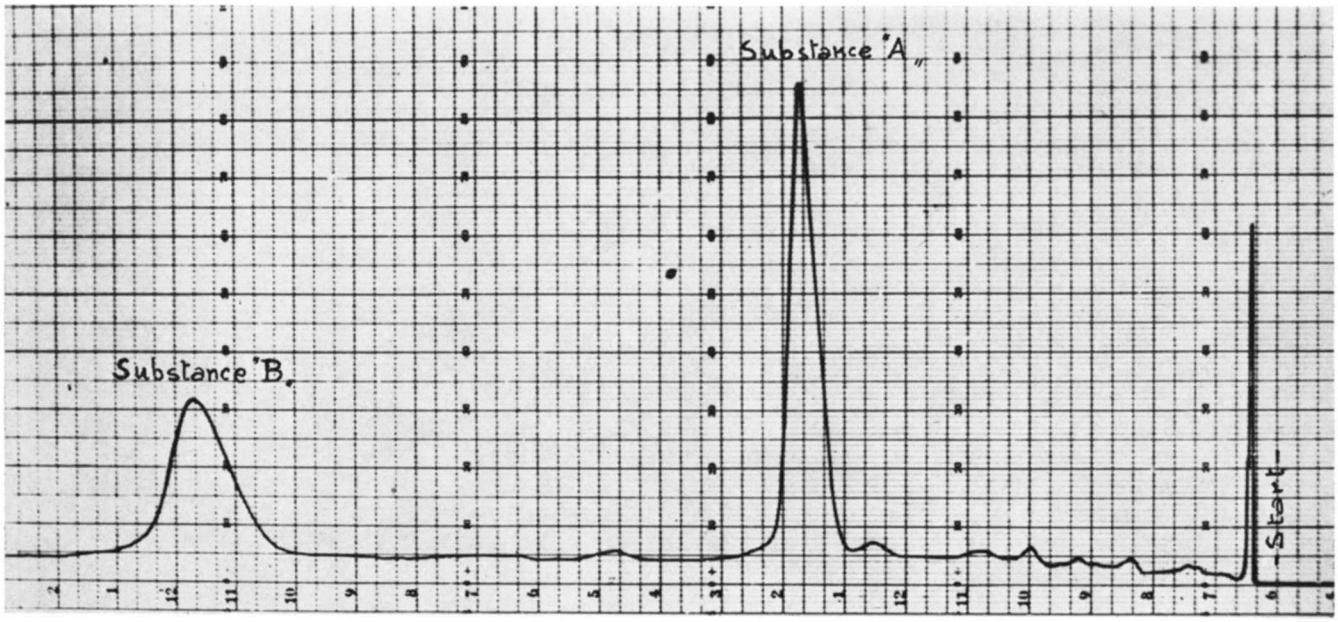


FIG. 1. GLC of the distillate on SE-30. Substance A was identified as phytol, substance B as geranyl geraniol.

with sulfuric acid or spraying with 2,7-dichlorofluorescein (sodium salt) in alcohol.

Gas–Liquid Chromatography. Both polar and nonpolar columns were used. The nonpolar column was used in a Fractovap model C-AID/F chromatograph (Erba, Milan) with flame ionization detector. It was 2 m × 4 mm I.D. and contained SE-30 (methylpolysiloxy gum, General Electric), 1% on silanized Gas-Chrom P, 100–200 mesh, at 190°C. The flash heater was at 250°C and the carrier gas was nitrogen, 40 ml/min.

The polar column, in a Fractovap model B with thermal conductivity detector, contained ethylene glycol succinate polyester, 20% on Chromosorb W, 60–80 mesh. Column, 210°C. Flash heater, 250°C. Carrier gas, helium, 4.5 liters/hr.

Infrared spectra were recorded on a Perkin-Elmer Infracord apparatus.

RESULTS

Linseed oil, 25 kg, was molecularly distilled at 240°C and 1.5 × 10^{-5} mm pressure; the distillate (250 g), containing 20–25% unsaponifiable material, was saponified. For the fractionation, the unsaponifiable fraction was subdivided in 30-g portions and each portion chromatographed on 1 kg of alumina in a column, 55 mm I.D.

Petroleum ether (bp 60–80°C) (4 liters) eluted 2.97 g of polyenic hydrocarbons (fractions 1–4). The study of this fraction has been previously reported in a note (10). Further elution with 15 liters of benzene–petroleum ether 1:1 gave 12.78 g of a slightly colored solid (yellow-reddish Liebermann-Burchard reaction) (fractions 5–18), and finally elution with 15 liters of benzene yielded 15.2

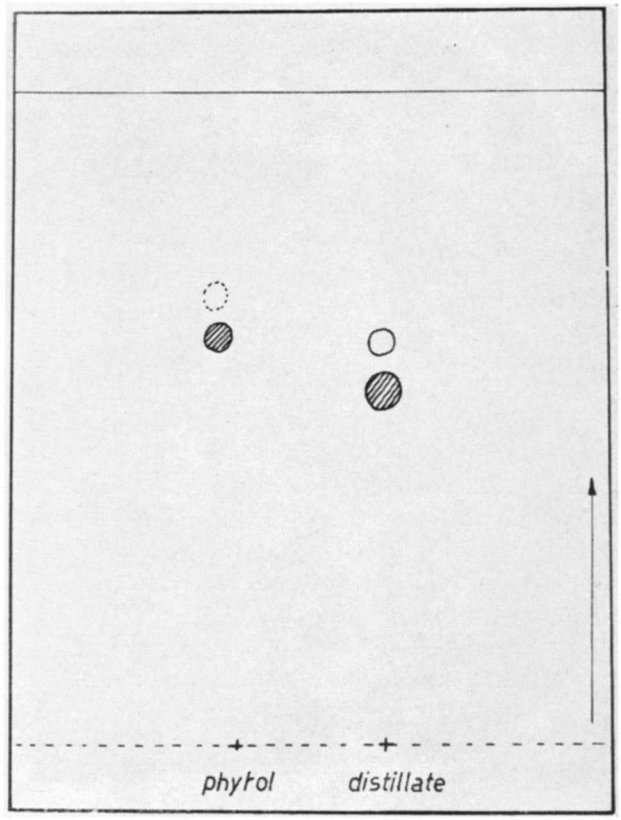


FIG. 2. Fractionation of the mixture of substance A and B by TLC on Silica Gel G with hexane–ethyl acetate 3:1. Substance A has the higher R_f, equal to that of phytol. Detected with iodine vapor.

g of sterols (fractions 19–33). The composition of this last fraction has been described earlier (11).

Fractions 5–18 were crystallized from acetone; the small amount of waxy solid obtained consisted mainly of aliphatic alcohols. The residue after evaporation of the mother liquor and treatment with methanol gave an abundant precipitate of pure cycloartenol (12); by distillation of the mother liquor at 3 mm a volatile fraction, bp 150–200°C (0.71 g), and a residue (1.47 g) were obtained. GLC of the distillate on polar (see Fig. 1) and nonpolar columns showed the presence of two main components besides smaller quantities of more volatile products. These two components could be separated by TLC in petroleum ether–ethyl acetate 3:1 (Fig. 2); 400 mg of the distillate was therefore placed on a column (8 mm I.D.) filled with 12 g of silicic acid and eluted with hexane–diethyl ether 19:1. The 15 fractions obtained were examined by TLC and GLC, and suitably combined to give substance A, with the smaller retention volume, refractive index $[n]_D^{25} = 1.4621$, and substance B, $[n]_D^{25} = 1.4910$.

Substance A

The infrared spectrum (Fig. 3) indicated the probable presence of a primary α,β-unsaturated, β-substituted OH group (band 1000 cm^{-1}); bands at 1375 and 735 cm^{-1} indicated the presence of isopropyl and trimethylene groups respectively.

Substance A was hydrogenated in methanol in the presence of Adam's catalyst; 100 mg adsorbed 7.52 ml of hydrogen under normal conditions (expected, 7.5 ml for one double bond). The infrared spectrum of hydrogenated A showed a shift of the 1000 cm^{-1} (C—O stretching of the α,β-unsaturated primary alcohol) band to 1050^{-1} (C—O stretching of a normal saturated primary alcohol).

Analysis: $C_{20}H_{42}O$; calculated: C, 80.46; H, 14.18
found: C, 80.74; H, 14.21

These findings suggested that substance A might be phytol; the identity was proved by comparing both IR spectra and GLC retention volumes of substance A and of its hydrogenated derivative with those of authentic samples of phytol and dihydrophytol.

Substance B

The IR spectrum (Fig. 4) suggested that a primary α,β-unsaturated, β-substituted OH group was present (band at 1000 cm^{-1}); the 835 cm^{-1} band could be attributed to an isopropenyl group.

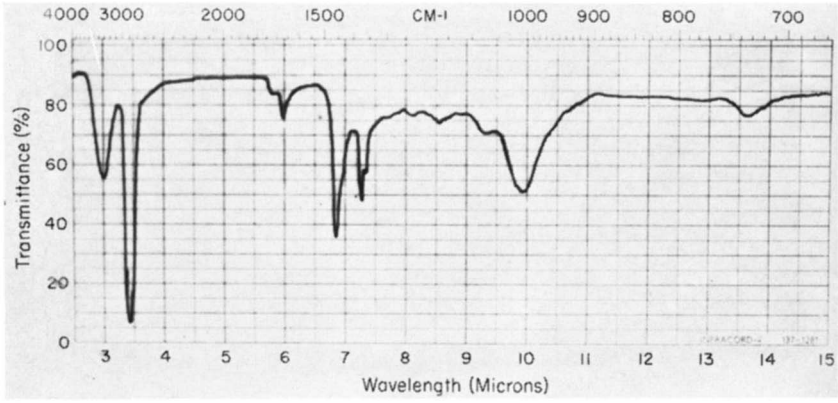


FIG. 3. IR spectrum of substance A (phytol).

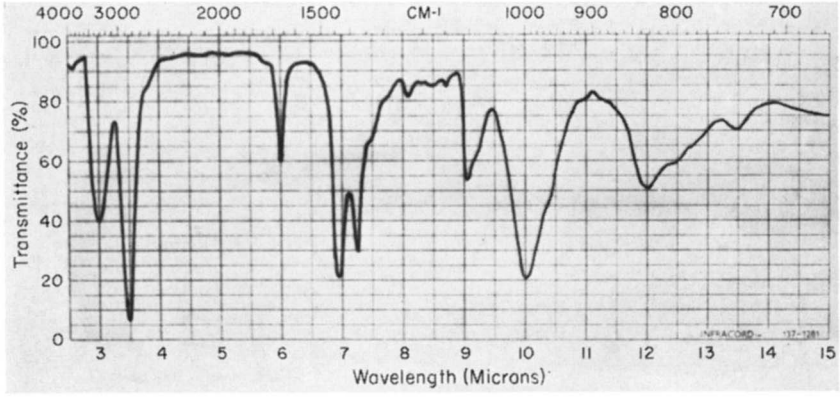


FIG. 4. IR spectrum of substance B (geranyl geraniol).

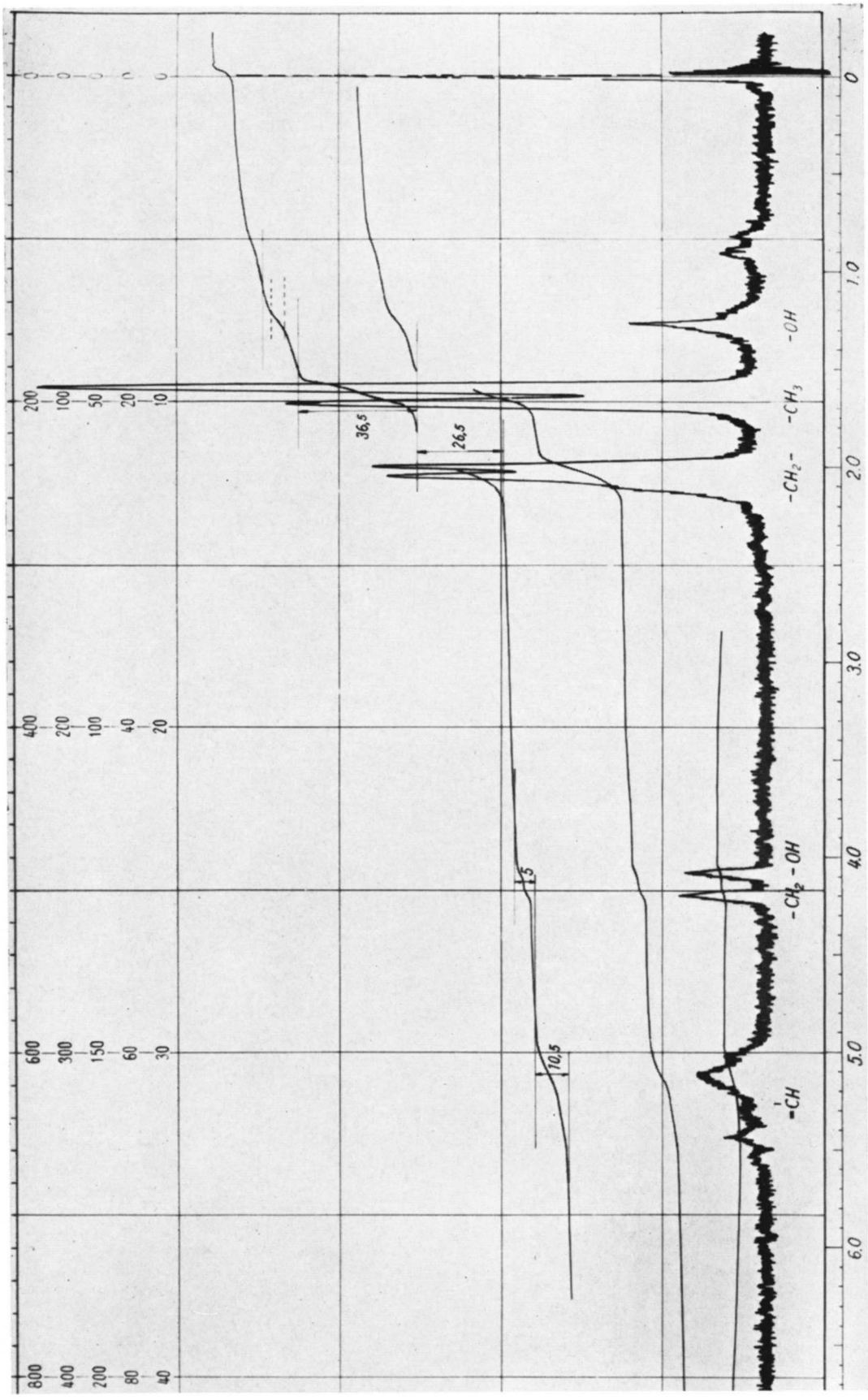


FIG. 5. NMR spectrum of substance B (geranyl geraniol).

TABLE 1

Type of H	Chemical Shift (ppm)	Farnesol		Calculated for Geranyl Geraniol	Found for Substance B
		Calculated	Found		
—OH	variable	1	1.08	1	*
—CH_2—OH	4.15	2	2.00	2	2
—C=CH—	4.90–5.70	3	3	4	4.2
—CH_2—	1.80–2.20	8	6.86	12	10.6
—CH_3	1.50–1.80	12	11.0	15	14.6

* Not calculated because impurities absorb in the region of the —OH band.

By catalytic hydrogenation (24.4 ml of hydrogen per 100 mg of product) a compound was obtained with $[n]_D^{25} = 1.4610$. The IR spectrum of the hydrogenated product was identical with that of dihydrophytol. GLC on the polar column unexpectedly showed five peaks, one of which corresponded to dihydrophytol.

The results so far obtained indicated that substance B was a diterpenic alcohol with the same type of skeleton as phytol but different from it in molecular weight and in the number of double bonds. Although hydrogenation yielded at least five compounds, the purity of substance B could not be questioned, since it showed a single peak on GLC, on both polar and nonpolar column. Concluding that B could be the C_{20} homologue of farnesol, i.e., geranyl geraniol, we compared its IR spectrum with that of farnesol. They were very similar. The conclusion is supported by a comparison between the nuclear magnetic resonance spectrum of substance B (Fig. 5) and that of farnesol. Calculated and found NMR values for the two compounds are shown in Table 1.

The mass spectrum of substance B was too complex to be interpreted fully, but the molecular weight of 290 deduced from it supported the proposed geranyl geraniol formula ($C_{20}H_{34}O$); a peak at 290 − 18 = 278 is probably attributable to a dehydration product.

Chemical confirmation of the proposed structure for substance B was obtained as follows. Acetyl farnesol and acetylated substance B were ozonized in pentane with a titrated solution of ozone. The ozonides were reduced with hydrogenated Lindlar catalyst and the products were converted to their 2,4-dinitrophenylhydrazones. Farnesol and substance B both yielded a mixture of derivatives, from both of which was obtained by crystallization a 2,4-dinitrophenylhydrazone, mp 231–233°C. These derivatives had identical IR spectra and were identified as levulinic aldehyde DNP (mp 231–234.5°C). Analysis confirmed this finding.

Analysis: $C_{17}H_{16}O_8N_8$;

calculated: C, 44.33; H, 30.50; N, 24.35

found: C, 44.30; H, 30.70; N, 24.12

From the mother liquors the 2,4-DNP of acetone was crystallized, but in neither case could the expected acetoxyacetaldehyde 2,4-DNP be detected.

From the evidence so far obtained we conclude that substance B from unsaponifiable fractions of linseed and peanut oils is geranyl geraniol. The only doubtful point appears to be the mixture of five different compounds obtained by hydrogenation, starting from a substance which appeared to be a single substance according to GLC. A possible explanation would be that a number of isomers are formed as a result of hydrogenation at asymmetric centers and that the isomers are separable by GLC. As far as we know, this is the first time that phytol has been identified in a fatty oil and that geranyl geraniol has been isolated from a natural source.

This study is part of Research Project FG-It-105, sponsored by the U.S. Department of Agriculture. We wish to renew our thanks to the U.S.D.A. for the authorization to publish the work.

Manuscript received 23 November 1965; accepted 10 February 1966.

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