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Constituents from Seeds of *Alpinia galanga* WILD. and Their Anti-ulcer Activities[1]

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The potent anti-ulcer principles, 1′-acetoxychavicol acetate (**1**) and 1′-acetoxyeugenol acetate (**2**), were isolated from seeds of *Alpinia galanga* WILD., and established by chemical syntheses.

Besides, three sesquiterpenes, caryophyllene oxide (**10**), caryophyllenol-I (**11**) and caryophyllenol-II (**13**), along with *n*-pentadecane, *n*-7-heptadecene and fatty acid methyl esters, were also isolated.

Methanolic extract of seeds of *Alpinia galanga* WILD. (Chinese name "Houdoukou," 紅豆蔻, Zingiberaceae), one of the stomachics in chinese drugs,[3] showed significant inhibitory activity against Shay ulcer in rats. We report herein structure elucidation and synthesis of the two effective α-vinyl benzyl alcohol derivatives, and refer to structures of essential oil components obtained from the extract.

Fractionation of the methanolic extract, guided by the assay against Shay ulcer in rats, revealed that the inhibitory activity was concentrated in the fraction containing ether soluble neutral substances. Further fractionation involving silica gel chromatography yielded the anti-ulcer substance **1** and **2** (Fig. 1).

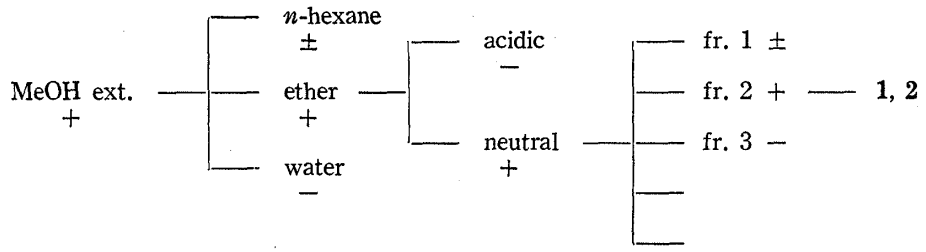


Fig. 1. Fractionation of Active Components guided by Anti-Shay Ulcer Assay (+ positive, ± weak and − negative)

These components manifested remarkable inhibitory activities to the ulcer as shown in Fig. 2, when given intraperitoneally to Shay rats at a dosage of 2 to 10 mg per kg.

The component **1**, $C_{13}H_{14}O_4$ (*m/e* 234, M^+), showed the nuclear magnetic resonance (NMR) signals due to two acetyl groups at δ 2.00 and 2.15, a terminal methylene group at 5.0—5.4, an olefinic proton at 5.7—6.1 and A_2B_2 system protons on an aromatic ring, besides doublet of one proton at δ 6.30 (Fig. 3). In addition to the NMR data, the infrared (IR) absorption bands at ν_{max} 1760, 1740, 1600, 1500 and 1200—1240 cm^{-1} suggested that **1** was 1′-acetoxychavicol acetate.

1) Presented at the Annual Meeting of the Japanese Society of Pharmacognosy, Nagasaki, Nov. 1972 (by A.O. and S.K.) and Osaka, Oct. 1974 (by H.N. and S.M.).
2) Location: *1-2-58, Hiromachi, Shinagawa-ku, Tokyo, 140, Japan.*
3) Institute of Medicines, Chinese Medical Society Academy (ed.), "Record of Chinese Herbal Medicines," (中葯志, in Chinese), Vol. II, National Health Publisher, Peking, 1961, p. 126.

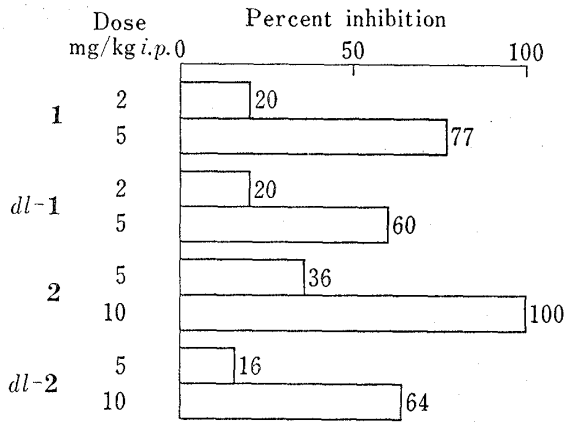


Fig. 2. Anti-Shay Ulcer Effect in Rats by Native (**1** and **2**) and Synthetic (*dl*-**1**=*dl*-1′-Acetoxychavicol Acetate and *dl*-**2**=*dl*-1′-Acetoxyeugenol Acetate) Compounds

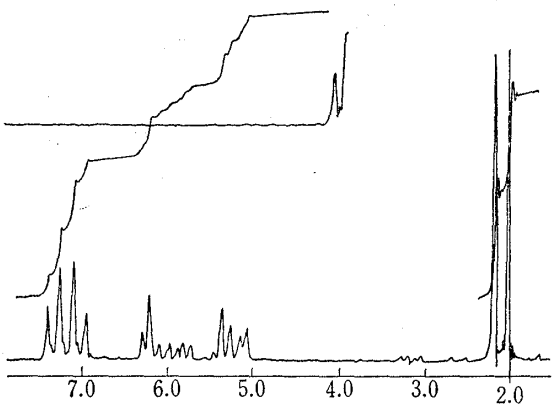
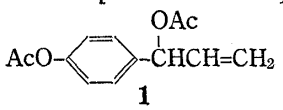


Fig. 3. NMR Spectrum of Compound **1**



1

Hydrogenation of **1** on paladium-charcoal giving *p*-propyl-phenol acetate (**3**) supports this structure. Since the optical rotation of the dihydro compound (**4**) prepared by hydrogenation of **1** on Adams catalyst indicated the value of $[\alpha]_D^{20}$ −91.7°, the natural product (**1**) must be *S*-configuration[4] (Chart 1).

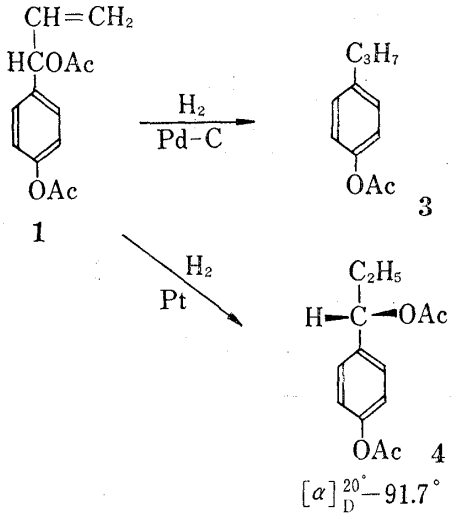


Chart 1

Another anti-Shay ulcer substance (**2**), $C_{14}H_{16}O_5$ (*m/e* 264, M^+), showed very similar NMR spectrum except a singlet at 3.85 ppm due to a methoxy group and ABX pattern signals assignable to the 1,2,4-trisubstituted benzene ring. Comparing the mass spectral patterns of both compounds clarified the structure of **2** as shown in Fig. 4, in which the peaks at 163 and 162 may be ascribed to the fragment ions noted, respectively.

Structures of these biological active components were established by the unambiguous syntheses as follows. Grignard reaction of *p*-hydroxybenzaldehyde (**5**) with two moles of vinylmagnesium bromide followed by acetylation gave *dl*-1′-acetoxychavicol acetate (**6**) whose spectra were identical with those of natural product (**1**). Treatment of vanillin acetate (**7**) with vinylmagnesium bromide yielded 4-acetoxy-3-methoxy-α-vinylbenzyl alcohol (**8**) which was acetylated by usual manner giving the desired com ound, *dl*-1′-acetoxyeugenol acetate (**9**) (Chart 2).

Anti-Shay ulcer effects of the synthetic compounds were also shown in Fig. 2. Moreover, *dl*-1′-acetoxychavicol acetate depressed significantly the gastric secretion in Shay rats even at 2 mg per kg as shown in Fig. 5. This is of interest in connection with the anti-ulcer activity.

Interestingly, these anti-ulcer compounds **1** and **2** have the characteristic taste of the chinese drug "Houdoukou."

Apart from the above biological active substances, four sesquiterpenes (**a**, **b**, **c-1** and **c-2**) were isolated from the hexane soluble part (Fig. 1) by repeated column chromatographies, together with hydrocarbons and fatty acid methyl esters, which were in detail described in experimental.

4) R. MacLead, F.J. Welch, and H.S. Mosher, *J. Am. Chem. Soc.*, **82**, 876 (1960).

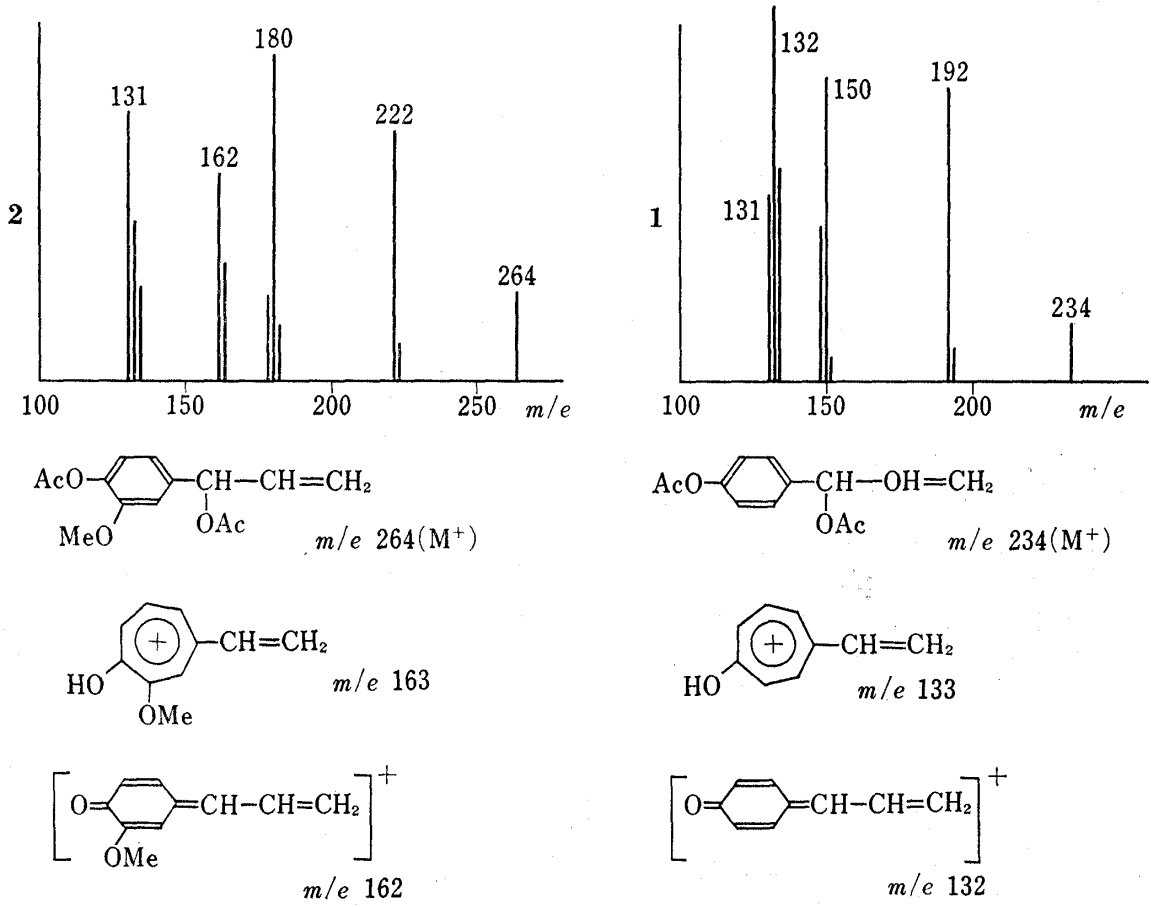


Fig. 4. Mass Spectra of Compounds **1** and **2**

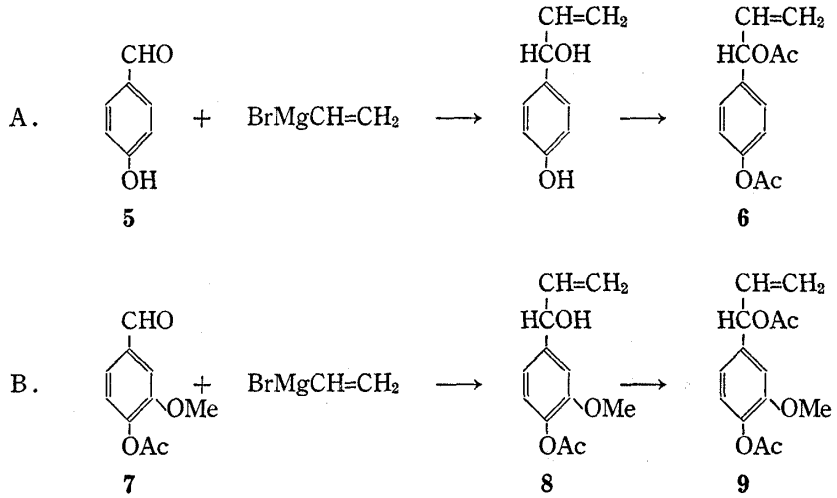


Chart 2

The spectroscopic data of a major sesquiterpene **b**, mp 58—61°, $[\alpha]_D^{20}$ −59° (c=2.7, $CHCl_3$), $C_{15}H_{24}O$ (m/e 220, M^+), were suggestive of caryophyllene oxide (**10**).

This was confirmed by direct comparison with the authentic sample[5] through gas-liquid chromatography (GLC).

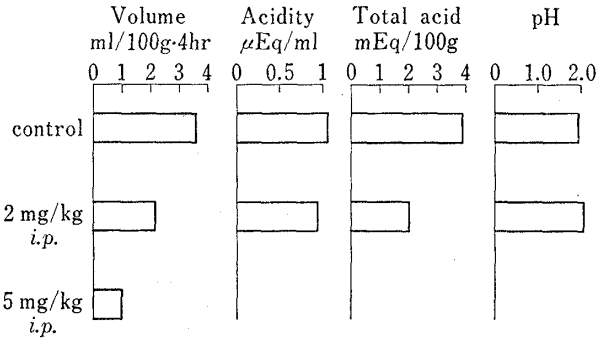


Fig. 5. Influence of *dl*-1′-Acetoxychavicol Acetate on Gastric Secretion in Shay Rats

Other sesquiterpene **c-1**, $[\alpha]_D$ −52.1° (c=0.21, $CHCl_3$), $C_{15}H_{24}O$ (m/e 220, M^+), and **c-2**, $[\alpha]_D$+133.7° (c=0.30, $CHCl_3$), $C_{15}H_{24}O$ (m/e 220, M^+), indicated analogous spectral data in NMR, IR and mass spectra, but differed from each other in retention time (t_R value in minute) on GLC and the value of optical rotation. The NMR spectrum of **c-2** showed signals for tertiary methyl groups at δ 0.94 and 0.97, one vinyl methyl group at 1.58 (d, J=1 Hz), one hydroxyl group at 1.78, one terminal methylene group at 4.42 and 4.67, one hydroxy-methine at 4.78 and one olefinic proton at 5.47.

Treatment of caryophyllene oxide (**10**) with pyridinium hydrobromide[6] afforded caryophyllenol-I[7] (**11**), having 6α hydroxyl group and agreeing with t_R value of sesquiterpene **c-1** on GLC analysis. Since the oxidation products of the caryophyllenol-I (**11**) and sesquiterpene **c-2** were identical by means of GLC, **c-2** could be elucidated as caryophyllenol-II (**13**) (Chart 3).

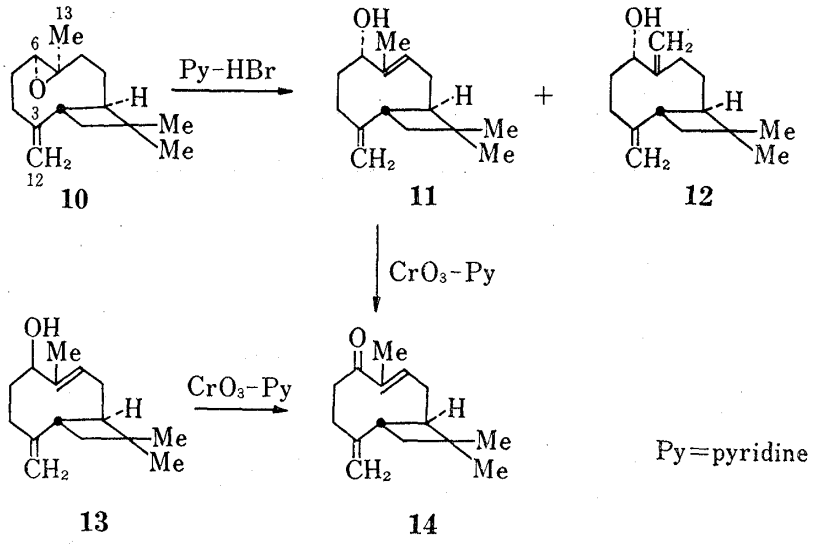


Chart 3

On the basis of the above results, **c-1**, and **c-2** could be assigned to caryophyllenol-I and caryophyllenol-II, respectively, which had been found in *Dipterocarpus pilosus* Roxb.[7b]

5) The authors are very grateful to Dr. A.S. Gupta of National Chemical Laboratory (India) for kindly providing us with a sample of authentic natural caryophyllene oxide and physical data of caryophyllenol-II.

6) *a*) M. Holub, V. Herout, M. Horak, and F. Sorm, *Collection Czech. Chem. Commun.*, **24**, 3730 (1959); *b*) G. Delle Monache, I.L. D'Albuguerque, F. Delle Monache, G.B. Marini Bettolo, and G.M. Nano, *Tetrahedron Letters*, **1971**, 659.

7) *a*) N.P. Damodaran and S. Dev, *Tetrahedron*, **24**, 4123 (1968); *b*) A.S. Gupta and S. Dev, *ibid.*, **27**, 635 (1971); *c*) K.H. Schulte-Elte and G. Ohloff, *Helv. Chim. Acta*, **51**, 494 (1968).

Experimental[8]

Bioassay——Anti-ulcer activity was tested in Shay rats.[9] Male Donryu rats, weighing 130—150 g and fasted for 48 hr, underwent ligation of the pylorus under ether anesthesia and were killed 18 hr after ligation. Through macroscopic examination of the stomach, the severity of the lesions of the forestomach was classified according to an arbitrary scale (0—5).[10] The test sample was intraperitoneally administered immediately after ligation.

Anti-gastric secretory effects were also studied in Shay rats. The animals were killed 4 hr after ligation. Gastric juice was collected and the volume, pH, acidity (determined by titration with 0.1 N NaOH to pH 7) and total acid were determined. The test sample was intraperitoneally administered immediately after ligation.

Extraction and Ether Soluble Fraction——Dried seeds (5.2 kg) of *Alpinia galanga* WILD. were extracted with hot MeOH. The methanolic extract was concentrated and taken up with *n*-hexane (hexane) and ether successively. The ether soluble neutral fraction (120 g), washed with 5% $NaHCO_3$, was chromatographed on silica gel (4.5 kg) using benzene as eluent. Each fraction was checked by bioassay against Shay ulcer in rats to isolate 5 g of the anti-ulcer substance **1** and 3 g of **2**.

1: $[\alpha]_D^{20}$ −80° (c=1.0, EtOH), IR ν_{max}^{liq} cm^{-1}: 1760, 1740, 1600, 1500, 1200—1240. NMR (CCl_4) δ: 2.00 (3H, s), 2.15 (3H, s), 5.0—5.4 (2H, m), 5.7—6.1 (1H, m), 6.36 (1H, d), 6.9—7.5 (4H, q). Mass Spectrum *m/e*: 234 (M^+, $C_{13}H_{14}O_4$), 192, 150, 133, 132, 131.

Anal. Calcd. for $C_{13}H_{14}O_4$: C, 66.65; H, 6.02. Found: C, 66.59; H, 6.06.

2: IR ν_{max}^{liq} cm^{-1}: 1760, 1740, 1600, 1500, 1200—1240. NMR (CCl_4) δ: 2.11 (3H, s), 2.30 (3H, s), 3.85 (3H, s), 5.1—5.2 (2H, m), 5.6—6.2 (1H, m), 7.0—7.6 (3H, m). Mass Spectrum *m/e*: 264 (M^+, $C_{14}H_{16}O_5$), 222, 180, 163, 162, 133, 132, 131. *Anal.* Calcd. for $C_{14}H_{16}O_5$: C, 63.62; H, 6.10. Found: C, 63.57; H, 6.11.

Hydrogenation of 1——A solution of **1** (100 mg) in EtOH was hydrogenated over Pd-C (20 mg) by usual method. Filtration and evaporation of the solvent gave 75 mg of *p*-propylphenol acetate (**3**); IR ν_{max}^{liq} cm^{-1}: 1760, 1500, 1360, and 1210—1190, NMR (CCl_4) δ: 0.87 (3H, t), 1.57 (2H, m), 2.13 (3H, s), 2.53 (2H, t) and 6.8—7.2 (4H, br. q).

A solution of **1** (100 mg) in EtOH was shaken under hydrogen atmosphere using Adams catalyst (5 mg) and the hydrogenation was stopped after one mole equivalent of hydrogen was absorbed. Usual work-up gave 83 mg of the dihydro compound (**4**); $[\alpha]_D^{20}$ −91.7° (c=0.5, EtOH), IR ν_{max}^{liq} cm^{-1}: 1750, 1730, 1600, 1500, 1360 and 1230—1190, NMR (CCl_4) δ: 0.83 (3H, t), 1.73 (2H, m), 1.95 (3H, s), 2.15 (3H, s), 5.60 (1H, t), and 6.8—7.3 (4H, q) (Chart 1).

Synthesis of *dl*-1′-Acetoxychavicol Acetate (6)——To a solution of vinylmagnesium bromide prepared from magnesium (2.4 g) and vinyl bromide (10.7 g) in dry tetrahydrofuran (THF) (50 ml), was added a solution of 6.0 g of *p*-hydroxybenzaldehyde (**5**) in THF (20 ml) under ice-cooling. After the reaction mixture was stored for 3 hr at room temperature, aqueous NH_4Cl solution was added. The oily product obtained by usual work-up was acetylated with acetic anhydride (5 ml) in pyridine (15 ml) followed by purification using silica gel column chromatography giving 5.8 g of *dl*-1′-acetoxychavicol acetate (**6**), whose spectral data were completely coincided with those of the natural product (**1**).

Synthesis of *dl*-1′-Acetoxyeugenol Acetate (9)——To a solution of 9.5 g of vanilline acetate (**7**) in dry THF (50 ml) was added a solution of 0.05 M vinylmagnesium bromide in THF (25 ml) under ice-cooling under nitrogen. After stirring for 3 hr at room temperature, the reaction mixture was treated with saturated aqueous NH_4Cl solution. Acetylation of the product (**8**) and purification of the acetate using silica gel column chromatography gave 8.0 g of *dl*-1′-acetoxyeugenol acetate (**9**) which was identified with the natural product (**2**) by spectroscopic measurement.

Hexane Soluble Fraction——The hexane soluble part (52.2 g) fractionated from the same MeOH extract was chromatographed on silica gel (1.62 kg) column; fraction H-1 (2.7 g) was obtained from hexane–benzene

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8) The following instruments were used for obtaining the physical data. Melting points: Yamato Melting Point Apparatus Type MP-1 and recorded uncorrectedly; Specific Rotations: Perkin-Elmer Model 141 automatic polarimeter in 1 dm tubes; IR spectra: Perkin-Elmer Model 221 or Perkin-Elmer Infracord; NMR spectra: Varian A-60 spectrometer (tetramethylsilane as an internal standard); Mass spectra: JEOL JMS-01SG Spectrometer; GC-MS: JEOL JMS-D-100 (using 4% OV-1 on stainless steel column, 3 feet length and 1/8 inch OD.); GLC: Hewlett-Packard 5711A (using glass column, 3 feet length and 1/8 inch OD., DEGS=diethylene glycol succinate polyester and column temperature was recorded.). Thin-layer chromatography (TLC) was carried out on TLC-plates Silica gel 60 F_{254} (E. Merck). Preparative TLC was performed by use of PLC-plates Silica gel 60 F_{254} (thickness 2 mm, E. Merck). For column chromatography, silica gel 60—110 mesh (Kanto Chemical Co., Inc., Tokyo) and aluminiumoxide (Art. 1097, E. Merck) were used. Colouring reagent: Iodine vapour or Vanillin-Sulphuric acid (after J.S. Mattew, *Biochim. Biophys. Acta*, **69**, 163 (1963).

9) H. Shay, S.A. Komarov, S.S. Fels, D. Meroze, M. Gruenstein, and H. Siplet, *Gastroenterology*, **5**, 43 (1945).

10) E. Adami, E. Marazzi-Uberti, and C. Turba, *Arch. int. Pharmacodyn.*, **147**, 113 (1964).

(9: 1) eluate, H-2 (5.4 g) from (5: 5) and H-3 (21.3 g) from benzene–ether (9: 1—8: 2) successively. The other resinous matter (18 g) from benzene–ether (7: 3—5: 5) eluate remains untried.

Fraction H-1——By means of GC-MS analysis on OV-1 at 120°, the colourless liquor (2.7 g) was comprised of *n*-pentadecane, t_R=2.5 min ($C_{15}H_{32}$, M+ *m/e* 212), and *n*-heptadecene, t_R=5.7 min ($C_{17}H_{34}$, M+ *m/e* 238).

In order to determine the olefinic position of *n*-heptadecene, fraction H-1 (240 mg) was treated with alkaline permanganate at 75° in the usual way.[11] The acidic oil (8.3 mg) from the reaction mixture was treated with diazomethane and was subjected to GC-MS analysis (30% DEGS at 120°), resulting in a mixture of methyl *n*-heptanoate (t_R=3.0 min, $C_8H_{16}O_2$, M+ *m/e* 144) and methyl *n*-decanoate (t_R=5.5 min, $C_{11}H_{22}O_2$, M+ *m/e* 186). Consequently, the *n*-heptadecene was proved to be *n*-7-heptadecene.

Fraction H-2——The oily material (5.4 g) was a mixture of methyl esters of palmitic (t_R=5.5 min, 34% in relative quantity of this fraction), stearic (t_R=2.0 min, 4%), oleic (t_R=13.1 min, 56%) and linoleic (t_R=17.8 min, 6%) acids by the use of GLC analysis on 30% DEGS at 150°.

Fraction H-3——Eight g of this fraction (21.3 g) mentioned above was chromatographed over silica gel (250 g) column using benzene–EtOAc as solvent. The early 5% EtOAc (in benzene) eluate gave an oily residue (518 mg, sesquiterpene **a**), the second matter (570 mg, sesquiterpene **b**) from subsequent 5% and 10% EtOAc eluate and the third (1.78 g, sesquiterpene **c**) from 15% EtOAc eluate.

Sesquiterpene a——After partial purification of crude **a** (518 mg) by preparative TLC using benzene as solvent, a viscous material (140 mg) eluted from the plates was further chromatographed on $AgNO_3$–silica gel[12] (140 g) column, giving an oily material (14.6 mg) with ultimate purity from petroleum benzine–benzene (7: 3) eluate.

Its spectral data were shown as follows; IR ν_{max}^{liq} cm^{-1}: 1715, 1640, 1280, 1120, 1070, 890, 740, Mass Spectrum *m/e*: 260 (M+, $C_{17}H_{24}O_2$), 245, 232, 217, 205. These data are suggestive of an acetylated sesquiterpene, but the chemical structure remains still obscure.

Sesquiterpene b——The crude material (570 mg) was rechromatographed over silica gel (30 g) column using benzene as solvent, and followed by preparative TLC with benzene. A residue eluted from the chromatoplates was treated with MeOH to give a crystalline matter (152 mg), mp 58—61°, $[\alpha]_D$ −59° (*c*=2.7, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1635, 1260, 1080, 960, 915, 895, 870, 765. NMR ($CDCl_3$) δ: 0.98 (6H, s, geminal methyl), 1.18 (3H, s, methyl on an oxirane ring), 2.87 (1H, dd, *J*=10.5 and 3.5 Hz, proton on an oxirane ring), 4.88 and 4.98 (2H, terminal methylene).

Mass Spectrum *m/e*: 220 (M+, $C_{15}H_{24}O$), 205, 202, 187, 177. *Anal.* Calcd. for $C_{15}H_{24}O$: C, 81.76; H, 11.20. Found: C, 81.59; H, 11.29. The t_R value (4.0 min) of this material on GLC over 30% DEGS at 150° was in complete agreement with that of the authentic caryophyllene oxide (**10**).

Sesquiterpene c——The crude oily residue (1.78 g) described above was rechromatographed over aluminium oxide (activity II—III, 88 g) column to give a material (260 mg) from 20% EtOAc in hexane eluate. Then, it was further chromatographed over $AgNO_3$–silica gel (20 g) column with 10% benzene in hexane under GLC (30% DEGS at 150°) monitoring to give two oily materials, **c-1** (5.5 mg); t_R=13.0 min, $[\alpha]_D$ −52.1° (*c*=0.21, $CHCl_3$)[15] and **c-2** (52 mg), t_R=15.0 min, $[\alpha]_D$+133.7° (*c*=0.30, $CHCl_3$).[15] IR spectra of **c-1** and **c-2**; ν_{max}^{liq} cm^{-1}: 3280, 1620, 1010, 880.

Mass Spectra of **c-1** and **c-2**; *m/e* 220 (M+, $C_{15}H_{24}O$), 202, 187, 177, 159. The NMR spectrum of **c-2**; δ: 0.94 (3H, s, tertiary methyl), 0.97 (3H, s, tertiary methyl), 1.58 (3H, *J*=1.0 Hz, olefinic methyl), 1.78 (1H, s, hydroxyl group which disappears by deuteration), 4.42 and 4.67 (2H, terminal methylene), 4.78 (1H, br. m, olefinic proton).

Isomerization of Caryophyllene Oxide——Caryophyllene oxide (**10**, 57 mg) in dry pyridine (2.0 ml) was heated in the presence of pyridinium hydrobromide (75 mg) under reflux for 1 hr. After cooling and filtration, an oily material (24.5 mg) was obtained from the filtrate by usual work-up. The product was comprised of two main compounds, caryophylla-3(12),7(13)-dien-6α-ol (t_R=11 min, **12**) and caryophylla-3(12),7-dien-6α-ol (t_R=13 min, **11**), that is caryophyllenol-I, by the use of GLC (DEGS, 150°).

Ketones (14) from Caryophyllenol-I and c-2——To CrO_3-pyridine complex (CrO_3 79 mg in 1 ml of dry pyridine) was added the above isomerized product (6 mg) in pyridine (1 ml) and stirred at room temperature for 3 hr. The dark reaction mixture was poured into ice-cold 1 N H_2SO_4 (10 ml) and worked up by extraction with ether in the usual manner, giving an oily material (3 mg, ν_{max}^{liq} 1680 cm^{-1}).

On GLC over DEGS at 120° it could be separated into caryophylla-3(12),7(13)-dien-6-one (t_R=16.2 min) and caryophylla-3(12), 7-dien-6-one (t_R=19.7 min, **14**).

Sesquiterpene **c-2** (6 mg) was also oxidized in the same manner to convert into a ketone (2.7 mg), which was 19.7 min in t_R value of the GLC analysis.

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11) J.W. Hill and W.L. McEwen, "Organic Syntheses," Coll. Vol. II, ed. by A.H. Blatt, John Wiley and Sons, Inc., New York, N.Y., 1943, p. 53.
12) A.S. Gupta and S. Dev, *J. Chromatog.*, **12**, 189 (1963).
15) Samples for measurement of optical rotation were further purified by preparative TLC on $AgNO_3$–silica gel plates (D.R. Idler and L.M. Safe, *Steroids*, **19**, 315 (1972)).