Isolation and Structure of Sesangolin, a Constituent of *Sesamum angolense* (Welw.)

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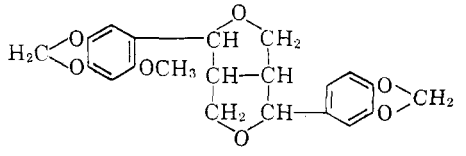
The seed oil of the wild sesame plant *Sesamum angolense* is unusually high in synergistic activity with pyrethrum. The oil was shown to contain two synergists, sesamolin and a previously undescribed compound that we have named sesangolin. Analysis of the latter indicated the presence of one methoxyl, two methylenedioxyphenyls, and two benzyl ether groups. Aliphatic double bonds, C-methyl groups, and acetal structures (aside from those on the methylenedioxyphenyl groups) were absent. Permanganate oxidation yielded piperonylic and 6-methoxypiperonylic acids. Nitric acid oxidation yielded the dextrorotatory di-γ-lactone of α,β-bis(hydroxymethyl)succinic acid. Based on these fragments sesangolin is 2-(3,4-methylenedioxyphenyl)-6-(6-methoxy-3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]octane and its bridgehead hydrogens are in a *cis* configuration. Proton nuclear magnetic resonance spectra were especially useful in elucidating the structure of the compound and a detailed examination of the spectrum supports the assigned formula. Sesangolin is about equal to sesamin in ability to increase the insecticidal potency of pyrethrum.

An examination of the seed oil (sesame oil) from thirty-three different strains of *Sesamum indicum*[3] disclosed a maximum amount of *ca.* 1.5% of sesamin and sesamolin, the only ingredients of sesame oil known to be synergistic with the insecticide pyrethrum. The report of Pearman, *et al.*,[4] stating that a seed oil derived from a wild sesame plant of Northern Rhodesia, *Sesamum angolense* (Welw.), contained 9% sesamin (based on synergistic action with pyrethrins) was therefore of great interest, especially since such an oil may be a valuable source of synergistic materials. The high sesamin value, which was based on bioassay, may have been caused by the presence of sesamolin, a compound known to have a synergistic activity as much as five times that of sesamin.[5] Pearman, *et al.*, did not consider sesamolin as an ingredient because at the time of their work sesamolin was not known to be a synergistic component of sesame oil. They also reported that their oil contained 7.4% of unsaponifiables, a value obviously inconsistent with a 9% figure for the unsaponifiable sesamin. These considerations led us to investigate the constituents of the wild sesame oil.

The petroleum ether extract of *S. angolense* seed, upon removal of the solvent, yielded oil and crystals.[6] Analysis by the method of Suarez[7] of the oil made homogeneous by heating until the crystals dissolved indicated that it contained 3.73% sesamin and 2.69% sesamolin. This result confirmed that the percentage of synergistic constituents in the oil was indeed large (totaling 6.42%), but the sesamin content was much below the 9% value reported by Pearman. However, if the greater synergistic activity of sesamolin as compared with sesamin is taken into account, the high biological activity reported by Pearman, *et al.*, appears to be verified.

Because of the lack of specificity of Suarez's ultraviolet analysis (most compounds with the 3,4-methylenedioxyphenyl structure would absorb strongly in approximately the same wave length region) and the fact that the oil came from a new plant species, the isolation of the sesamin and sesamolin from the oil of *S. angolense* seed was attempted in order to prove their presence. Chromatography of the oil mixture on silicic acid gave two distinct zones. Although the first yielded sesamolin, the second did not give sesamin, but a compound different from either sesamin or sesamolin. No reference to a previous isolation of the compound could be found and accordingly the name sesangolin is proposed for it. Based on the experimental evidence, detailed below, sesangolin has the structure shown in formula I.



I

The synergistic action of sesangolin was found to equal that of sesamin, increasing the toxicity of pyrethrins to house flies approximately threefold in an equiproportional mixture.[8]

At first sesangolin melted at 87–88°, but in later experiments it melted at 101°. Cross-seeding experiments established the higher melting form as the more stable.

(1) Part of this work was submitted by W. A. Jones as M.S. thesis to the American University, Washington, D. C.

(2) National Institutes of Health, Bethesda, Maryland.

(3) M. Beroza and M. L. Kinman, *J. Am. Oil Chemists' Soc.*, **32**, 348 (1955).

(4) R. W. Pearman, W. D. Raymond, and J. A. Squires, *Colonial Plant Animal Prod.* (Gr. Brit.), **2**, 297 (1951).

(5) M. Beroza, *J. Am. Oil Chemists' Soc.*, **31**, 302 (1954).

(6) The oil was kindly supplied by Mr. G. D. Glynne Jones, Pyrethrum Board of Kenya, Nakuru, Kenya Colony, Africa.

(7) C. C. Suarez, R. T. O'Connor, E. T. Field, and W. G. Bickford, *Anal. Chem.*, **24**, 668 (1952).

(8) The tests against house flies were carried out by W. A. Gersdorff and P. G. Piquett, of the Entomology Research Division.

The purity of sesangolin was indicated by its sharp melting point, and by the single zone with constant ultraviolet absorption ratios[9] obtained for each of the fractions in the chromatography of the oil. The purity of sesangolin was further established by our inability to fractionate it by gas and paper chromatography.

Elemental analyses and molecular weight determinations pointed to $C_{21}H_{20}O_7$ as the formula of sesangolin. The compound contains one methoxyl group[10] but no carbon-linked methyl.[11] Since it did not decolorize potassium permanganate in acetone even when heated to 60° for several minutes, aliphatic double bonds are absent. A Villavecchia color test,[7] which indicates the presence of a 3,4-methylenedioxyphenyl acetal structure such as is present in sesamolin, was negative. No acetal structure (aside from those on the methylenedioxyphenyl groups) is present because sesangolin survived acid hydrolysis.

The hydrogenation of sesangolin in acetic acid with a palladium–charcoal catalyst resulted in the rapid uptake of two moles of hydrogen, after which the compound absorbed hydrogen very slowly. The rapid uptake of the two moles of hydrogen is consistent with the presence of the two benzyl ether groups shown in the sesangolin formula.

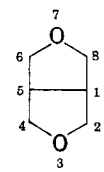
The nitration of sesangolin yielded a dinitro compound.

The ultraviolet spectrum of sesangolin is practically identical with that of sesamin and sesamolin,[12] when compared on a molar basis. The close similarity of the chromophoric groups of these compounds was therefore established and the presence of two methylenedioxyphenyl groups in the sesangolin molecule was strongly indicated. Methylenedioxyl analyses[13] further supported the presence of these groups.

The infrared spectrum of sesangolin provides additional evidence for the presence of methylenedioxyphenyl groups. A strong band at 1245 cm.$^{-1}$, characteristic of the methylenedioxyphenyl structure, is present. Another strong band, found at 1190 cm.$^{-1}$, has been associated with a methylenedioxyphenoxy group.[14] Model compounds containing this structure, 3,4 methylenedioxyphenyl propyl ether, 3,4-methylenedioxyphenyl butyl ether, 2-(3,4-methylenedioxyphenoxy)tetrahydropyran, 4-(2,3-dibromopropyl)-5-methoxy-1,2-methylenedioxybenzene, and sesamolin, all exhibited strong absorption in the 1175–1195-cm.$^{-1}$ region. Sesamin, which differs from sesamolin in possessing a methylenedioxyphenyl in place of a methylenedioxyphenoxy group, does not have a peak in this region. Inspection of the sesangolin formula shows that the methoxy-aryl group is a methylenedioxyphenoxy structure.

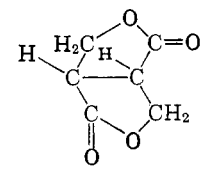
Sesangolin, when subjected to prolonged permanganate oxidation in refluxing acetone, yielded piperonylic and 6-methoxypiperonylic acid. The isolation of these acids from sesangolin confirms the presence in this molecule of one 3,4-methylenedioxyphenyl group and one 6-methoxy-3,4-methylenedioxyphenyl group, both attached to carbon atoms.

There remained only the problem of identifying the central nucleus which calculated by difference has a formula of $C_6H_8O_2$. This formula is identical with that of the central nucleus of sesamin, sesamolin, asarinin, pinoresinol, and eudesmin. These compounds are known to have the fused tetrahydrofuran structure shown in formula II with substituted phenyl groups at positions 2 and 6 (or 4 and 8). Nitric acid oxidation of these



II

compounds gives an optically active di-γ-lactone of α,β-bis(hydroxymethyl)succinic acid (III), the oxo groups fixing the carbon atoms to which the aryl



III

groups are attached. Erdtman and Gripenberg[15] have pointed out that the bridgehead hydrogens (1 and 5) must be in the *cis* configuration since a *trans* configuration of III would give a symmetrical molecule.

Nitric acid oxidation of sesangolin yielded the dilactone III, proving that the central nucleus of sesangolin is a 2,6-diaryl-3,7-dioxabicyclo[3.3.0]-octane structure with *cis* hydrogens at the 1- and 5-positions. The dilactone is dextrorotatory; the central nucleus of sesangolin has, therefore, the same configuration as that of sesamin and pinoresinol.

Proton nuclear magnetic resonance (n.m.r.) studies were especially useful in the complete structural elucidation and actually preceded some of the oxidation studies already described. Figure 1 shows the n.m.r. spectrum of sesangolin and for

(9) M. Beroza, *Anal. Chem.*, **22**, 1507 (1950).

(10) Method of E. P. Clark, "Semimicro Quantitative Organic Analysis," Academic Press, Inc., New York, N. Y., 1943.

(11) Method of W. F. Barthel and F. B. LaForge, *Ind. Eng. Chem.*, **16**, 434 (1944).

(12) P. Budowski, R. T. O'Connor, and E. T. Field, *J. Am. Oil Chemists' Soc.*, **28**, 51 (1951).

(13) M. Beroza, *Anal. Chem.*, **26**, 1970 (1954).

(14) M. Beroza, *J. Am. Chem. Soc.*, **77**, 3332 (1955).

(15) H. Erdtman and J. Gripenberg, *Acta Chim. Scand.*, **1**, 71 (1947).

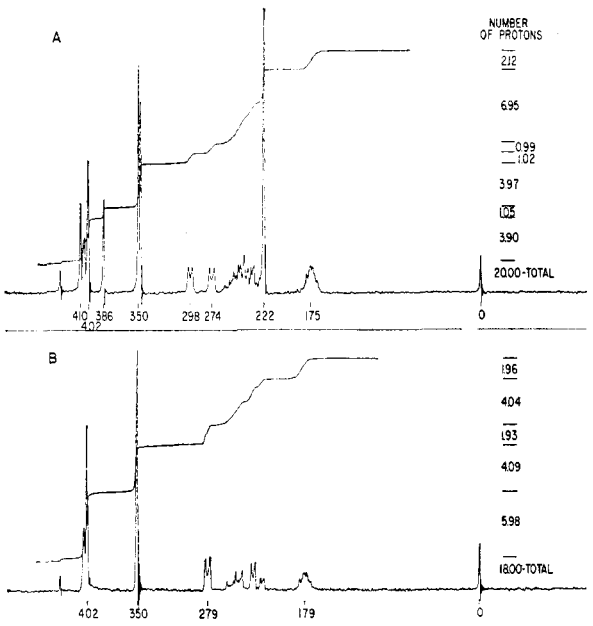


Fig. 1.—N.m.r. spectra of (A) sesangolin and (B) sesamin. Frequencies given in c.p.s. from internal tetramethylsilane.

comparison the spectrum of sesamin. Positions of lines (at 60 Mc.) are given in c.p.s. from internal tetramethylsilane. The lines at 348 and 350 are clearly ascribable to the two methylenedioxyl groups which are in slightly different chemical environments. The strong line at 222 is due to the methoxyl group, the chemical shift suggesting an aryl rather than alkyl ether (*e.g.*, Jackman[16] gives typical values of 224 and 197, respectively). The integral of the spectrum (Fig. 1) shows unquestionably that there are only five aromatic protons (in the region 386–410). Thus one of the methylenedioxyphenyl groups must be additionally substituted, presumably by the methoxyl group. The exact position of the methoxyl is established as follows: Comparison of the aromatic regions of sesangolin and sesamin (as well as other methylenedioxyphenyl compounds not shown here) indicates that the three aromatic protons in the *unsubstituted* methylenedioxyphenyl ring are responsible for the lines at 402 and 406. The lines at 386 and 410 are of approximately equal intensity, and the integral shows that the line at 386 is due to a single proton. The lines at 386 and 410 are thus established as arising from the two ring protons of the methoxyl-substituted methylenedioxyphenyl group. These protons must be *para* to each other since the lines are not split. Protons *ortho* or *meta* to each other are known to be coupled by approximately 8 and 3 c.p.s., respectively, whereas protons *para* to each other are coupled by less than 1 c.p.s.[17]

(16) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, p. 55.

(17) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, N. Y., 1959, p. 193.

The methoxyl group must therefore be in the position indicated in I. Isolation of 6-methoxypiperonylic acid from the oxidation of sesangolin (as described above) confirms this conclusion. The remaining features of the sesangolin spectrum are quite similar to those of sesamin, as indicated in Fig. 1. The unresolved multiplet centered at 175 is due to the two bridgehead protons. The unusual chemical shift for such protons on carbons β to an oxygen is apparently due to the fused tetrahydrofuran ring system. The complex group at *ca.* 220–270 is due to the two CH_2 groups α to the oxygens. The spin coupling in this group has not been analyzed.

The two doublets at 274 and 298 are clearly due to the protons α to both the oxygen and the phenyl groups. The 24 c.p.s. difference in their resonance frequencies indicates an appreciable chemical non-equivalence, whereas the corresponding protons in sesamin are equivalent, giving rise to a doublet at 279. The non-equivalence of these protons in sesangolin might result from the presence of the methoxy on one of the aromatic rings, but the difference seems large. Asarinin, the asymmetric stereoisomer of sesamin, has two doublets at 261 and 286, similar to sesangolin, but also shows resonance lines in the 190–220 region, where sesangolin and also sesamin have no lines. We believe that a more complete analysis of the spectra of sesangolin as well as of sesamin and its stereoisomers is needed before the n.m.r. data can furnish reliable information on the configuration of sesangolin.

The n.m.r. spectrum thus is seen to be in complete accord with formula I and in fact with the other evidence furnishes virtually conclusive proof of the correctness of this structure.

Experimental

Isolation of Sesangolin.—The initial isolation was accomplished essentially according to the method of Beroza.[18] A 2–3-g. sample of *S. angolense* oil was dissolved in 1:4 chloroform–isoöctane and introduced on an 80-g. silicic acid (Merck) column that had been prewashed with 100 ml. of 15% ethyl acetate in isoöctane and 75 ml. of isoöctane. The column was developed with 3% ethyl acetate in isoöctane, sesamolin being eluted between 700 and 1200 ml. and sesangolin between 1200 and 2000 ml. At 50-ml. intervals the absorption at 280, 288, and 300 mμ was determined in an ultraviolet spectrophotometer. The eluate from the first zone gave a 288/300-mμ absorbance ratio of approximately 2.0, a value in agreement with that of sesamolin. A white crystalline solid, subsequently isolated in 3.3% yield from this eluate, was determined to be sesamolin by m.p. (90–92°), mixed melting point with an authentic sample, infrared and ultraviolet spectra. The 288/300-mμ absorbance ratio of the eluate from the second zone was 1.2 (corresponding value of sesamin is 7.0). A white crystalline solid (sesangolin) melting at 87–88° after two crystallizations from methanol was obtained from this eluate; yield 3.3%.

Anal. Calcd. for $C_{21}H_{20}O_7$: C, 65.62; H, 5.24; mol. wt., 384. Found: C, 65.66, 65.43; H, 5.36, 5.33; mol. wt.

(18) M. Beroza, *Anal. Chem.*, **26**, 1173 (1954).

(Rast), 380; mol. wt. (Signer using methylene chloride),[10] 383.

In a mixture with sesamolin, sesangolin melted over a wide range, starting to melt at 74°.

To determine whether the solid crystalline residue that separated upon removal of solvent from a petroleum ether extract of *S. angolense* seed was largely sesangolin, the solid was filtered off, washed with isoöctane, and crystallized from absolute ethanol. The resulting crystals melted sharply at 101°. The identity of this compound was in doubt until its infrared spectrum was compared and found identical with that of sesangolin (m.p. 88°). Polymorphism was confirmed as follows: A small amount of the 101° material was dissolved in a minimum of hot alcohol and seeded with the 88° material. The crystals, which separated on cooling, melted at 101°. Similarly a crystal melting at 101° was used to seed the 88° material dissolved in a minimum of hot alcohol. It also produced crystals melting at 101°. Once the 101° material was obtained, the lower melting material could not be produced. The higher melting crystals therefore constitute the more stable form.

Sesangolin was also isolated most conveniently by the method of Tracy[19] for the extraction of pyrethrum synergists from sesame oil from a sample of *S. angolense* oil from which the solid had been removed: Distilled butyrolactone (1250 ml.) was added to 580 ml. of the oil in a 3-l., three-neck flask fitted with a thermometer, stirrer, condenser, and heating mantle. After heating the mixture with stirring at 150° for 2 hr., the mixture was allowed to come to room temperature while being stirred. The upper layer was separated and the butyrolactone distilled, leaving a residue that was taken up in petroleum ether (b.p. 60–70°). After removing the petroleum ether-soluble portion, the residue was crystallized three times from absolute alcohol and twice from amyl alcohol; yield was 5 g. of a product melting at 101°; $[\alpha]^{23}D$ +48.5° (*c* 0.54 chloroform). The Villavecchia color test was negative, showing that no sesamolin was present.

Gas Chromatography of Sesangolin.[20]—The gas chromatography was carried out at 180° on a 6-ft. column of Gaschrom P impregnated with 0.75% SE-30[21] using argon as the carrier gas with a flow rate of 15–17 cc./min. and an ionization unit as detector. A single symmetrical peak (other than that of the solvent) occurring at approximately 40 min. was obtained during an 80-min. run.

Paper Chromatography of Sesangolin.—This procedure, which identifies 3,4-methylenedioxyphenyl synergists by reversed-phase paper chromatography using 30% aqueous acetic acid as the developing solvent,[22] gave a single peak at R_f 0.54 for sesangolin.

Dinitrosesangolin.—A test tube containing 90 mg. of sesangolin in 0.6 ml. of glacial acetic acid was swirled in a 40° bath while 0.75 ml. of 2:3 by volume concentrated nitric–acetic acids was added dropwise over a 10-min. period. After another 5 min. in the bath the test tube was removed, cooled, and 5 ml. of water added with swirling. In a few minutes crystals began to form. They were filtered off, washed with water, dried, and recrystallized from absolute ethanol; wt. 28 mg., m.p. 153–154°.

Anal. Calcd. for $C_{21}H_{18}O_{11}N_2$: C, 53.17; H, 3.82; N, 5.91. Found: C, 53.28; H, 4.12; N, 5.08, 5.14.

Ultraviolet Spectrum of Sesangolin.—A solution containing 0.031 mg. per ml. of isoöctane exhibited peaks at 236 and 293 mμ. The molar extinction coefficients are: max. 293 mμ, 7870; min. 257 mμ, 622; max. 236 mμ, 8575; min. 222 mμ, 5830.

Infrared Spectrum of Sesangolin.—The infrared spectrum of a 1% solution (w./v.) of sesangolin in carbon disulfide (0.4-mm. light path) was determined with a Model 21 Perkin–Elmer spectrophotometer. Strong (s) and medium (m) peaks were observed at the following $cm.^{-1}$ values: 2850(m), 1245(s), 1190(s), 1155(m), 1040(s), 940(s), 860(m), 817 and 807 (m).

Nuclear Magnetic Resonance Studies.—The spectra were obtained with a Varian HR-60 spectrometer and V-3521 integrator using scan rates of 1–2 c.p.s./sec. for spectra and 7 c.p.s./sec. for integrals. Samples were dissolved in deuterochloroform at a concentration of about 10%, and tetramethylsilane was used as an internal reference. Line positions were determined by interpolation between audio side bands, and should be accurate to 1–2 c.p.s. Reported values of the integrals are the average of five determinations.

Permanganate Oxidation of Sesangolin.—Ten grams of potassium permanganate was added gradually over an 8-hr. period to a refluxing solution of 1 g. of sesangolin in 50 ml. of acetone. After standing overnight at room temperature, the manganese dioxide was filtered off, and the acetone filtrate held aside. A hot water wash of the manganese dioxide gave an alkaline filtrate that was concentrated to a small volume and acidified with hydrochloric acid. The solution was extracted with chloroform several times; the chloroform extract was dried over sodium sulfate and evaporated, giving 28 mg. of solid material.

The acetone filtrate was evaporated and the residue taken up in 5% potassium hydroxide. The ether extract of this solution yielded 30 mg. of unchanged sesangolin. The aqueous layer was acidified with hydrochloric acid and extracted with ether, which upon evaporation yielded 32 mg. of oily residue, not readily characterizable.

The solid residue was chromatographed on silicic acid[23] yielding two crystalline compounds. The first (9.4 mg.) which melted at 226° after recrystallization from absolute ethanol, was shown to be piperonylic acid by a mixed melting point with an authentic sample, ultraviolet and infrared spectra, and thin-layer chromatography.[23] The second compound (1.7 mg.) was chromatographed again and identified as 6-methoxypiperonylic acid[24] by its ultraviolet and infrared spectra, and its R_f value and characteristic color with chromotropic acid–sulfuric acid spray on thin-layer chromatography.[23]

Dilactone from Sesangolin.—The procedure followed was that used by Beroza[14] for the preparation of the same dilactone from sesamolin; yield from 1 g. of sesangolin was 39 mg. The crystals from benzene were recrystallized finally from absolute ethanol, m.p. 159–160° $[\alpha]^{23}D$ +184° (*c* 0.23 water).

Anal. Calcd. for $C_8H_6O_4$: C, 50.7; H, 4.3. Found: C, 51.35, 51.10; H, 4.54, 4.70.

The melting point of the dilactone was undepressed in admixture with the dilactone obtained from sesamin by following the same preparative procedure.

(19) R. L. Tracy, U. S. Patent 2,837,534 (June 3, 1958).

(20) Kindly carried out by Dr. Henry M. Fales of the National Heart Institute, Bethesda, Maryland.

(21) Applied Science Laboratories, Inc., State College, Pennsylvania.

(22) M. Beroza, *Anal. Chem.*, **28**, 1550 (1956).

(23) M. Beroza and W. A. Jones, *Anal. Chem.*, **34**, 1029 (1962).

(24) R. T. Arnold and N. Bortnick, *J. Am. Chem. Soc.*, **67**, 1797 (1945).