2-Butyl Propenyl Disulfides from Asafetida: Separation, Characterization, and Absolute Configuration*

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2-Butyl propenyl disulfide, the major volatile sulfur compound of asafetida oil, is separated into E- and Z-isomers by pressure liquid chromatography; the E,Z-ratio is 7:3.

(R)-Configuration of the predominant, levorotatory enantiomers and an enantiomeric purity of about 75 % of the natural disulfides in the present study follow from a described, enantiospecific synthesis of a 47:53 mixture of levorotatory E- and Z-isomers of (R)-2-butyl propenyl disulfide with an enantiomeric purity supposedly exceeding 93 %, departing from (S)-2-butanol.

A possible terpenoid origin of the disulfides is briefly discussed.

Asafetida, the oleogum resin derived from certain *Ferula* species, such as *F. alliacea* Boiss., *F. foetida* Regel, and *F. narthex* Boiss., native to Iran and Afghanistan, contains volatile sulfur compounds, the composition, quantities and properties of which have been studied in connexion with food problems (*cf.* Ref. 1 and literature cited therein). The characteristic smell of asafetida is largely due to volatile disulfides with a long chemical history. In 1936, Mannich and Fresenius [2] identified the levorotatory, major disulfide, $C_7H_{14}S_2$, previously described by Semmler,[3] as 2-butyl propenyl disulfide *1*, of unspecified stereochemistry. Recently, a similar disulfide fraction, obtained from an asafetida of Afghan origin ('Patheni


Et–CH(Me)–S–S–CH=CH–Me

1

MeS–CH(Et)–S–S–CH=CH–Me

2

Et–CH(Me)–S–S–CH₂–CH=CH–Me

3
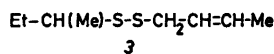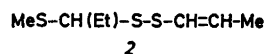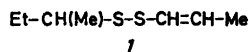
Hing'), was established as a levorotatory mixture of the E- and Z-isomers of *1*, with the former predominating.[4,5] A second, and minor, constituent $C_7H_{14}S_3$, was identified, here [5] and elsewhere,[4] as an E,Z-mixture of the disulfide *2*. Finally, a third sulfur component, $C_8H_{16}S_3$, was recognized as the allylic disulfide *3*, again occurring as a mixture of diastereomers.[4] Chemical syntheses of racemic E,Z-mixtures of *1*,[6] as well as of *2* and *3*,[7] have been recently reported.

Marked differences in the optical rotation of the major disulfide *1* obtained from gum resins of different origin, combined with uncertainty as to its biogenetic derivation, chirality, and propensity to undergo stereomutation, prompted the present, more detailed investigation, aimed at the separation of the various stereoisomers of *1*. The study was conducted on the levorotatory major fraction of the essential oil of asafetida ('Hing oil'), isolated and separated by inverted dry column and liquid chromatography. The distilled fraction (b.p. 105 °C/30 mmHg), consisting, according to GLC and ¹H NMR-spectroscopy, of 62 %
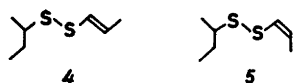
of the *E*-isomer *4*, 28 % of the *Z*-isomer *5*, and 10 % of a third compound, probably a monosulfoxide, exhibited physical data, including optical rotation, which differed significantly from those previously reported for similar fractions.[2-4] No isomerization was observable on distillation or GLC-analysis. Upon pressure liquid chromatography, the mixture was separated into virtually homogeneous *E*-isomer *4*, and a specimen of the *Z*-isomer *5*, containing *ca.* 10 % of *4* as the sole contaminant. Physical data and ¹H NMR-characteristics for the isomers are summarized in Table 1.

In order to establish the chirality and optical purity of *4* and *5*, a chemical synthesis of a mixture of the two was performed, departing from (*S*)-2-butanol and following the route designed for preparation of the diastereomeric

Table 1. Physical data and ¹H NMR characteristics for the *E*- and *Z*-isomers of 2-butyl propenyl disulfide (*4* and *5*).

$$H_3C - CH_2 - \underset{\underset{CH_3}{|}}{\overset{E}{CH}} - S - S - CH=CH - CH_3$$

| A | C | B | | 1 | 2 | 3 |
| | | | | G | F | D |

E-isomer (*4*).
$[\alpha]_D^{20}$ −38.4° (*c* 0.95, EtOH);[a] n_D^{25} 1.5153.[a] (Found: C 51.6; H 8.75: S 39.0. Calc. for $C_7H_{14}S_2$: C 51.8; H 8.69; S 39.5.)
¹H NMR (CDCl₃): δ 0.98 (A, t, *J* 7 Hz), 1.31 (B, d, *J* 7 Hz), 1.59 (C, m), 1.77 (D, d, *J* 5 Hz), 2.82 (E, m),[b] 5.89 (F, d, *J* 15 Hz), 6.10 (G, d, *J* 15 Hz).
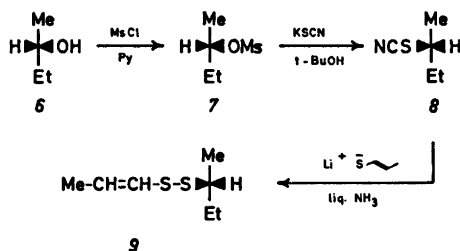
Z-isomer (*5*) (containing 10 % of *4*).
$[\alpha]_D^{20}$ −33.8° (*c* 0.85, EtOH);[a] n_D^{25} 1.516.[a,c] (Found: C 51.6; H 8.96; S 39.0.)
¹H NMR (CDCl₃): δ 0.98 (A, t, *J* 7 Hz), 1.31 (B, d, *J* 7 Hz), 1.59 (C, m), 1.77 (D, dd, $J_{3,2}$ 7 Hz, $J_{3,1}$ 1.5 Hz), 2.79 (E, m),[b] 5.67 F, 2q, $J_{2,1}$ 9 Hz, $J_{2,3}$ 7 Hz), 6.10 (G, dd, $J_{3,2}$ 9 Hz, $J_{1,3}$ 1.5 Hz).

[a] Reported for natural mixture of *4* and *5*: $[\alpha]_D^{25}$ −15.95° (*c* 1.25, EtOH),[4] n_D^{22} 1.5343.[4] [b] Reported (CCl₄): δ 2.73 (s),[6] and (CDCl₃): δ 2.29 (sext.).[4] [c] Determined by extrapolation to 100 % *5*.

racemates.[6] (*S*)-2-Butanol *6*, with an enantiomeric purity > 93 %, was converted into the mesylate *7* in a yield of 80 %, *i.e.* considerably higher than the 50 % previously reported for the production of the enantiomeric mesylate under different conditions.[8] In a carefully conducted displacement reaction (see Experimental), accompanied by inversion,[9] the mes-

ylate *7* afforded a 65 % yield of distilled (*R*)-2-butyl thiocyanate *8*, a sensitive compound undergoing partial isomerization to the isothiocyanate as well as further decomposition on storing. Its chemical and enantiomeric purity appears superior to that of a previously described specimen.[9] Conversion of *8* into a mixture of the *E*- and *Z*-isomers of (*R*)-2-butyl propenyl disulfide *9* was accomplished essentially as described for the racemic series.[6] According to GC-analysis, the *E*,*Z*-ratio of the synthetic product was 47:53, significantly smaller than that prevailing in the naturally derived mixture (*ca.* 70:30). The specific rotation of a 47:53 *E*:*Z*-mixture of the latter, with a value of −36°, calculated on the basis of the specific rotation of the individual isomers (Table 1), was significantly lower than the −46°, observed for the synthetic mixture with the same composition. On the reasonable assumptions (i) that the enantiomeric purity of the (*S*)-2-butanol used in the synthesis in fact exceeds 93 %, and (ii) that all steps in the sequence *6→9* proceed with complete stereochemical integrity, an expected maximum rotation of −49° for the mixture permits calculation of the specific rotation of the homogeneous (*R*),*E*- and (*R*),*Z*-2-butyl propenyl disulfides, as −52 and −46° (in EtOH), respectively. Hence, the enantiomeric purity of the natural *E*- and *Z*-isomers in the present study did not exceed 75 %. Since racemization during isolation can be precluded, the large differences in rotation values for

2-butyl propenyl disulfide reported in the various asafetida studies [α $-12.5°$ (neat),[3] α $-17.62°$ (neat),[2] and $[\alpha]_D^{25}$ $-15.95°$ (EtOH)[4]], suggest that the enantiomeric composition may vary greatly with the origin of the gum resin.

The above assignment of (R)-chirality to the levorotatory E- and Z-2-butyl propenyl disulfide is in keeping with the previously described formation of levorotatory 2-butanethiol ($[\alpha]_D^{25}$ $-14.6°$) upon reduction of the naturally derived ($-$)-disulfide(s) with zinc,[2] combined with the recent finding that (S)-2-butanethiol is dextrorotatory, with a reported $[\alpha]_D^{25}$ of $+34.44°$ for a specimen considered enantiomerically homogeneous.[10]

The (R)-chirality, predominant in the disulfides of natural origin, renders isoleucine, with its ($3S$)-configuration, an unlikely biogenetic donor of the 2-butyl grouping. By way of conjecture, an *in vivo* derivation from terpenoid progenitors appears conceivable, especially in view of the substantial quantities of monoterpenes, such as α-pinene, β-pinene, and, possibly, ocimene and alloocimene, encountered in this laboratory in the essential oil from asafetida gum resins.[5]

EXPERIMENTAL

Gas chromatography was performed on an F & M 810 instrument equipped with FID and packed columns (3 mm, 180 cm) containing OV-225 on Chromosorb G, or OV-17 on Chromosorb W as the stationary phase; carrier gas: He (20 ml/min); injection port: 250 °C; column temperature: isothermal in the range 90—120 °C. ^{1}H NMR spectra were recorded on a 90 MHz Bruker HX-90E instrument. Microanalyses were performed by Mr. G. Cornali and his staff.

Asafetida samples. TLC and GC analysis of the essential oil, procured from a variety of asafetida specimens of Afghan and Iranian origin, revealed the presence of from four to eight individual compounds. Two of these were disulfides, present in all samples, while a third disulfide only occurred in some of the specimens. The present study was conducted on asafetida samples of the Khadda (Iranian) or Hadda (Afghan) type, containing all three disulfides.

Isolation and fractionation of the volatile oils. The inverted dry column chromatographic technique [11] (silica gel: TLC grade, binder-free, NCL, Poona: 230 g. Distance run: 22 cm, solvent: hexane; mild suction (55—60 mmHg)) served to separate the crude oil (1.5 g), in

the course of 3.5 h, into three fractions: a, 100 mg; b, 90 mg; and c, 600 mg. Each of these was further purified by column chromatography (silica gel, hexane), followed by vacuum distillation, b.p.: a, 130 °C/4 mmHg, b, 125 °C/10 mmHg, and c, 105 °C/30 mmHg.

Separation of E- and Z-2-butyl propenyl disulfide of asafetida oil. By GLC, ^{1}H NMR, and MS, the three fractions were found to consist of the disulfides *1*, *2*, and *3*, yet partly contaminated with each other and with unidentified impurities. The present work is concerned solely with the major disulfide fraction, b.p. 105 °C/30 mmHg, containing the disulfides *1*, n_D^{25} 1.5152 [lit. n_D^{22} 1.5343[4]]; $[\alpha]_D^{20}$ $-36.9°$ (c 1, EtOH) [lit. α $-12.5°$ neat;[3] α $-17.62°$ (neat),[2] and $[\alpha]_D^{25}$ $-15.95°$ (c 1.25, EtOH)[4]]. The UV-, MS-, and ^{1}H NMR spectra were virtually identical with those reported for an E,Z-mixture of *1*.[4] On GLC, using the OV-225 column, the mixture appeared as a simple two-component system, present in the ratio 70:30, the minor component having the smallest retention time. However, chromatography on the OV-17 column (100 °C isothermal) revealed the presence of about 10 % of a third component, with a slightly higher retention time than both isomers (see below).

Isolation of virtually homogeneous E- and Z-isomers from the mixture was achieved by pressure chromatography on silica gel (Merck, prepacked columns, size B, *ca.* 2 ato), with petroleum ether (<60 °C) as the solvent, the intermediate fractions, containing more than one constituent, being recycled. After one passage, the major component, identified as the E-isomer by ^{1}H NMR, was obtained in a purity of >99 %, whereas the Z-isomer, even after a second column passage, contained about 10 % of the E-isomer according to GLC. Analytical specimens were produced by distillation at 1 mmHg onto a 'cold finger'. Analytical data, $[\alpha]_D^{25}$, n_D^{25}, and ^{1}H NMR patterns for the two isomers are presented in Table 1. Only minor differences were noted in their ^{13}C spectra.

Combined GLC/MS served to establish the identity of the third component in the mixture as one or more monosulfoxides, probably with the general structure Et$-$CH(Me)$-$S$-$S(O)$-$CH$=$CH$-$Me, supported by a molecular ion at m/e 178, accompanied by abundant fragment ions at m/e 122 (loss of butene) and at m/e 89 ([S(O)$-$CH$=$CH$-$Me]$^+$), corresponding to m/e 106 and 73 ([S$-$CH$=$CH$-$Me]$^+$) in the disulfide. To what extent the sulfoxide is a genuine constituent, or an artefact, remains unknown.

(S)-2-Butyl methanesulfonate 7. (S)-2-Butanol ($[\alpha]_D^{27}$ $+12.48°$ (neat); highest reported rotation: $[\alpha]_D^{27}$ $+13.52°$ (neat)[12]) (4.1 g), produced by resolution of the racemic alcohol, *via* the acid phthalate, with brucine,[12,13] was mixed with methanesulfonyl chloride (7.9 g) and cooled to 0 °C. During 15 min, 40 ml of an-

hydrous pyridine was added and the mixture was kept at $0-5$ °C for 2.5 h, before it was poured into ice-cold 10 % HCl (180 ml). The mesylate was extracted with ether. After drying and removal of the solvent, the mesylate (6.76 g $\sim$ 80 %) remained as a colourless oil exhibiting the expected ^{1}H NMR-spectrum. An analytical specimen was produced by distillation, b.p. $39-40$ °C at 1 mmHg, n_D^{25} 1.4237, $[\alpha]_D^{20}$ $+17.8°$ (c 2.1, CHCl$_3$) (Lit.[8] n_D^{25} 1.4232; $[\alpha]_D^{25}$ $-17.35°$ (neat).

(R)-*2-Butyl thiocyanate 8.* After much experimentation the following synthetic procedure was found useful: (S)-2-butyl mesylate (6.7 g) and KSCN (dried at 90 °C) (6.4 g) was heated to reflux in anhydrous *t*-BuOH (100 ml) for 1.5 h. After cooling, hexane (400 ml) was added and the azeotropic *t*-BuOH-hexane mixture (22:78), b.p. 63.7 °C, distilled slowly off from the mixture through a short column, care being taken not to expose the distillation flask to undue heating. When nearly all solvent had been removed, the thiocyanate was distilled, b.p. 23 °C at 1 mmHg. Water (about 3 ml) and ether were added to the distillate, which, according to ^{1}H NMR, still contained some butanol. The organic phase was thoroughly treated with CaCl$_2$ and the ether was removed at 0 °C *in vacuo*, leaving the thiocyanate (3.3 g$\sim$65 %) in virtually pure form. An analytical specimen was produced by shortway distillation at 1 mmHg, n_D^{25} 1.4589, $[\alpha]_D^{20}$ $-60.0°$ (c 2.4, EtOH) (Found: C 51.7; H 8.00; N 11.6; S 27.5, Calc. for C$_5$H$_9$NS: C 52.1; H 7.87; N 12.1; S 27.8). (Lit. n_D^{25} 1.4621; $[\alpha]_D^{20}$ $-24.98°$ (c 5.003, EtOH)).[9]

E(R)- *and* Z(R)-*2-Butyl propenyl disulfide 9.* (R)-2-Butyl thiocyanate (2.5 g) was brought into reaction, in liquid NH$_3$, with lithium propenylthiolate, prepared from Li (0.3 g) and ethyl propenyl sulfide [14] (2.2 g, 3:2 ratio of stereoisomers), as described for the racemic series.[6] The distilled reaction product (2.5 g $\sim$ 72 %) contained trace amounts of ethyl propenyl sulfide and 2-butyl thiocyanate in addition to the isomeric disulfides. The former could be removed on pressure liquid chromatography. A specimen, thus purified, consisted of 47 % *E*- and 53 % *Z*-isomer, according to GLC, n_D^{25} 1.5166 (Lit.[6] n_D^{20} 1.5172), $[\alpha]_D^{20}$ $-46°$ (c 1.6, EtOH). Control chromatography of the individual *E*- and *Z*-isomers revealed no tendency to equilibration.

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