Identification and Synthesis of (*Z*)-(1′*S*,3′*R*,4′*S*)(−)-2-(3′,4′-Epoxy-4′-methylcyclohexyl)-6-methylhepta-2,5-diene, the Sex Pheromone of the Southern Green Stinkbug, *Nezara viridula* (L.)

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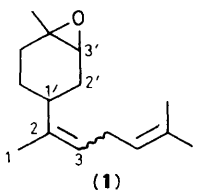
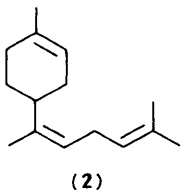
The sex pheromone of the male green stinkbug, *Nezara viridula* (L.) has been shown to be a novel epoxybisabolene (*Z*)-(1′*S*,3′*R*,4′*S*)(−)-2-(3′,4′-epoxy-4′-methylcyclohexyl)-6-methylhepta-2,5-diene, whose structure has been confirmed by spectroscopic studies and synthesis of the eight possible stereoisomers.

The southern green stinkbug, *Nezara viridula* (L.) is distributed throughout the tropical and neotropical regions of the world and is a major pest of cotton, citrus, cereal, and vegetable crops.[1] The defence secretion of *N. viridula* (L.) has been isolated and comprises largely n-tridecane and (*E*)-hex-2-enal.[2] This secretion originates from the dorsal abdominal glands together with hexanal and hexanol.[3] Previous studies have shown that male *N. viridula* (L.) produce a sex pheromone that is highly attractive to females,[4] and the tachid parasite *Trichopoda pennipes* (F.).[5] We report the identification and synthesis of a novel sesquiterpene (**1**) which is the major component of the sex pheromone of *N. viridula* (L).

The volatile materials emitted by >7 day old, non-diapausing male (or female) *N. viridula* (L.) (from Brazil) were collected on an activated charcoal filter by aeration, and extracted with methylene chloride. A bioassay comparing the responses of females to live males or extracts was used to monitor the purification process.[6] The extract from aeration of males was highly attractive to females, eliciting a courtship ritual and rapid upwind approach to the odour source. Volatile materials from aerations of females were inactive. A sesquiterpene hydrocarbon, a mono-oxygenated sesquiterpene, n-dodecane, n-tridecane, and n-nonadecane were identified by g.c.–mass spectrometry of the male aeration extract. A large scale aeration of male *N. viridula* (L.) followed by h.p.l.c. separation (Zorbax O.D.S.; 1:4 water–methanol; pentane extraction) and subsequent preparative g.c. (FFAP 10%; 100 °C, 12 °C min^{-1} to 240 °C) afforded the purified mono-oxygenated sesquiterpene (3 mg); *m/z* 220 (M^+, 7%), 164(23), 131(17), 121(24), 109(99), 107(58), 93(66), 91(40), 82(63), 67(48), 55(48), 43(76), and 41(100); δ_H (360 MHz, $CDCl_3$) 1.1—1.5 (2H, m), 1.3 (3H, br.s), 1.55 (3H, br.s), 1.60 (3H, br.s), 1.65 (3H, br.s), 1.60—2.1 (4H, m), 2.65 (3H, m), 3.05 (1H, br.s), and 5.05 (2H, m); δ_C (90.6 MHz, $CDCl_3$) 17.8, 19.3, 24.7, 25.8, 26.4, 26.6, 29.5, 30.2, 30.4, 61.0, 123.6, 124.1, 131.5, and 137.8 (C-4′ not observed). The sample was biologically active at the 5—15 ng level. Consideration of this



(**1**)



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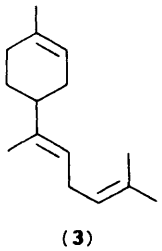

(**3**)

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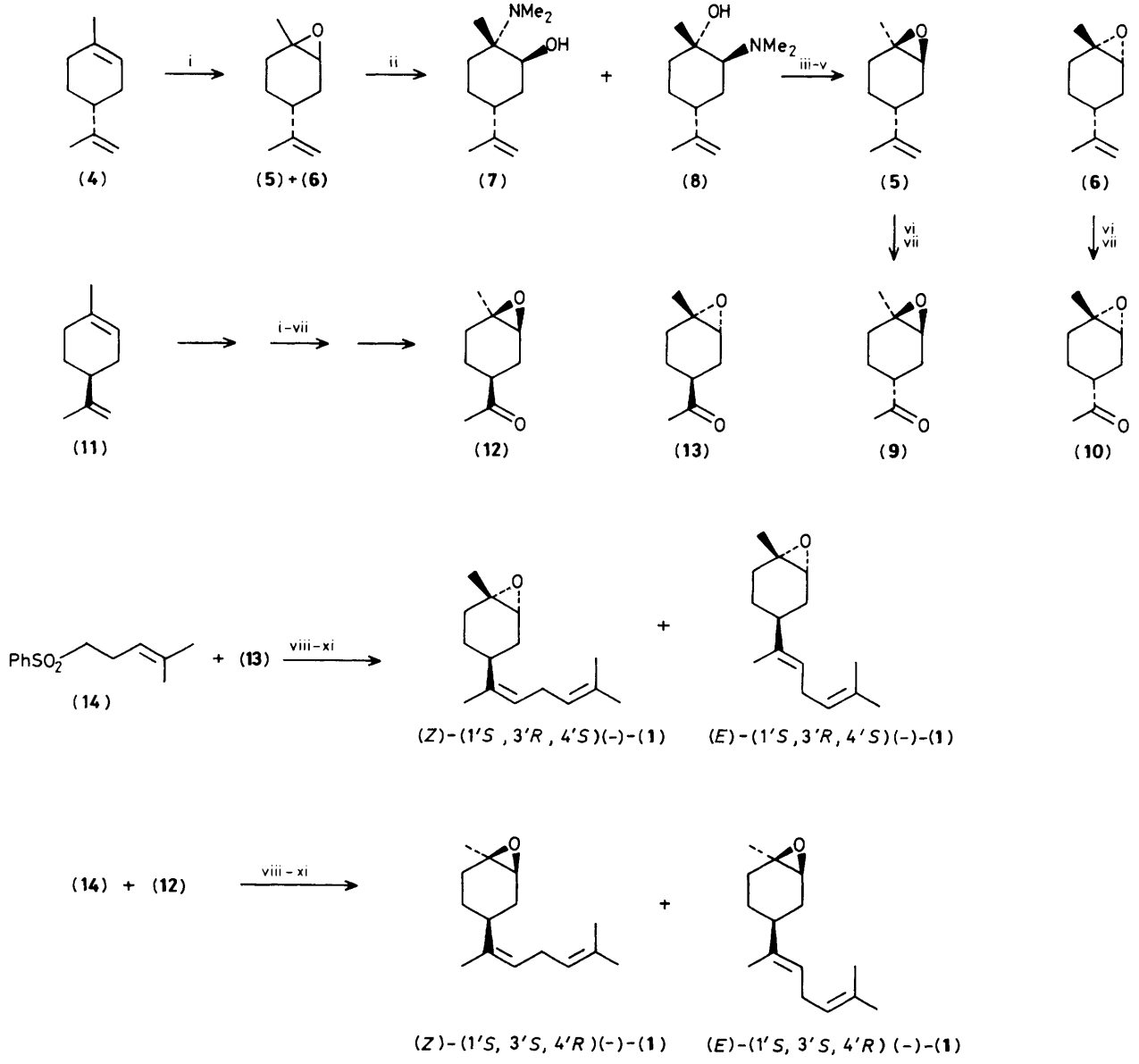
Table 1. Comparison of spectral and chromatographic data for the natural product and epoxybisabolenes.[a]

	C-1 δ	C-1′ δ	H-1′ δ	H-3′ δ	R_t/min,[b] g.c.	R_t/min,[c] h.p.l.c.
Natural product	19.3	30.4	2.65	3.05 br.s	22.8	14.8
(*Z*)-α-Bisabolene (**2**)	19.2	35.5	2.68	—	—	—
(*Z*)-(1′*S*,3′*R*,4′*S*)-(**1**)	19.3	30.6	2.63	3.0 br.s	22.8	14.8
(*Z*)-(1′*S*,3′*S*,4′*R*)-(**1**)	19.1	34.9	2.41	2.94 d	23.2	14.6
(*E*)-α-Bisabolene (**3**)	14.2	42.9	1.82—2.12	—	—	—
(*E*)-(1′*S*,3′*R*,4′*S*)-(**1**)	14.6	38.3	1.72—1.93	2.99 br.s	24.6	15.3
(*E*)-(1′*S*,3′*S*,4′*R*)-(**1**)	13.8	42.9	1.82	2.92 d	25.1	15.7

[a] Identical data were obtained for the other four enantiomers of (**1**). [b] G.c. on BP10, 25 m × 0.2 mm; 80 °C for 2 min then 6 °C min^{-1} to 180 °C. [c] H.p.l.c. on μ-Bondapak C18, 30 cm; eluant 1 : 4 water–acetonitrile.



Scheme 1. *Reagents:* i, *m*-$ClC_6H_4CO_3H$, 0 °C, CH_2Cl_2; ii, aq. Me_2NH, 150 °C; iii, separation; iv, MeI; v, aq. KOH; vi, O_3, −45 °C, CH_2Cl_2; vii, PPh_3; viii, Bu^nLi; ix, (**13**); x, PhCOCl; xi, 5% Na–Hg, tetrahydrofuran–MeOH.

spectral data led to the conclusion that the compound was an epoxybisabolene of general structure (**1**). In particular, the methyl resonance at δ 1.3 and the proton resonance at δ 3.05 suggested the methyl–epoxide arrangement, the three other methyl resonances and the proton signal at δ 2.65 indicated the skipped diene side-chain, and comparison of the ^{13}C data with data for (*Z*)- and (*E*)-α-bisabolene (**2**) and (**3**)[7] supported the proposed epoxybisabolene structure.

A synthesis of the eight stereoisomers of (**1**) was designed, beginning from a chiral precursor to avoid separation of enantiomers. Regioselective epoxidation[8] of (*R*)(+)-limonene (**4**) gave a mixture of epoxides (**5**) and (**6**) which were converted into three products (ratio 3.2 : 2 : 1) of which the two major ones were the aminoalcohols (**7**) and (**8**) (Scheme 1).[9] The aminoalcohols (**7**) and (**8**) were separated by recrystallisation of their tosyl salts, the methiodide salts were formed, and these were converted into enantiomerically pure limonene oxides (**5**) and (**6**) by treatment with base.[9] Ozonolysis afforded the epoxyketones (**9**) and (**10**).[10] A similar sequence starting from (*S*)(−)-limonene (**11**) gave the epoxyketones (**12**) and (**13**). A modified Julia reaction[11] of the sulphone (**14**) with *trans*-epoxyketone (**13**), followed by reductive elimination, afforded a 1 : 1 mixture of (*E*)- and (*Z*)-isomers of the corresponding epoxybisabolene (Scheme 1), as a colourless oil (~80%), the components of which were readily separable by h.p.l.c. (μ-Bondapak C18; 1 : 4 water–acetonitrile). The other six isomers were prepared in an analogous manner from epoxyketones (**9**), (**10**), and (**12**) [exemplified by reaction with *cis*-epoxyketone (**12**), Scheme 1]. Only the (*Z*)-(1′*S*,3′*R*,4′*S*)(−)-(**1**) ($[\alpha]_D$ −14.7°, *c* 1.2 in CH_2Cl_2) and (*Z*)-(1′*R*,3′*S*,4′*R*)(+)-(**1**) ($[\alpha]_D$ + 19.1°, *c* 0.53 in CH_2Cl_2) isomers had similar spectral data to the natural product, and in addition, δ_H (360 MHz, CD_2Cl_2) 5.05 (1H, t, *J* 7.4 Hz) and 5.07 (1H, t, *J* 7.4 Hz); δ_C (90.6 MHz, CD_2Cl_2) 57.2 (C-4′). The double bond geometry was unequivocally determined by proton nuclear Overhauser enhancements (n.O.e.) between the vinylic protons and adjacent methyl groups, two such enhancements being observed for the (*Z*)-double bond isomers but only one for the (*E*)-isomers. This correlated with ^{13}C shifts of C-1, C-1′, and H-1′ in the known (*Z*)- and (*E*)-α-bisabolenes (**2**) and (**3**)[7] (Table 1). The relative stereochemistry of the epoxide could be determined from the ^1H n.m.r. signal for H-3′ which was a doublet (*J* 4.5 Hz) at δ *ca.* 2.93 (H-3′ pseudoaxial) in the *cis*-epoxides [(*Z*)- and (*E*)-(1′*S*,3′*S*,4′*R*)(−)-(**1**) and their enantiomers], and a broad singlet at δ *ca.* 3.0 in the corresponding *trans*-epoxides (Table 1).[10] A comparison of the mass spectral, ^1H and ^{13}C n.m.r., and chromatographic data of the natural and synthetic products confirms the natural product to be either (*Z*)-(1′S,3′R,4′S)(−)-(**1**) or its enantiomer. Biological tests of the two pure enantiomers showed that only the (*Z*)-(1′*S*,3′*R*,4′*S*)-(−)-(**1**) compound produced behavioural responses in female *N. viridula* (L.) identical to that induced by live males, at a dose level of 12 ng.[6] The response of females to mixtures of the two enantiomers was linearly related to the concentration of the active enantiomer, and no synergism due to the inactive enantiomer was observed. The detailed behavioural responses to these compounds will be described more fully elsewhere. The work described was concerned with *N. viridula* (L.) from Brazil. A parallel study on *N. viridula* (L.) from France showed that both (*Z*)-diastereoisomers were present in the volatile materials emitted by males [2 : 1 ratio of *trans* : *cis* (*Z*)-3,4-epoxy-α-bisabolenes]. The absolute stereochemistry of these compounds has yet to be determined. In conclusion, the sex pheromone produced by male *N. viridula* (L.) has been demonstrated to be (*Z*)-(1′*S*,3′*R*,4′*S*)(−)-(**1**) by unambiguous chemical synthesis and behavioural studies.

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