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SYNTHESIS OF S-(+)-4-METHYL-3-HEPTANONE, THE PRINCIPAL ALARM PHEROMONE OF *ATTA TEXANA*, AND ITS ENANTIOMER

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Abstract—The principal alarm pheromone of *Atta texana*, S-(+)-4-methyl-3-heptanone, and its enantiomer have been prepared synthetically in high optical purity.

In the course of our studies of the alarm pheromone systems of fungus growing ants of the genus *Atta*, S-(+)-4-methyl-3-heptanone has been identified as the principal alarm pheromone of *Atta texana*, $[\alpha]_D^{25} = +22{\cdot}0° \pm 0{\cdot}4°$, and the same compound has been identified from another species, *Atta cephalotes*, $[\alpha]_D^{27} = +22{\cdot}0° \pm 0{\cdot}4°$.[1] The isolation, identification and biological activity of the ketone from *A. texana* and other species of *Atta* had been previously described[2,3] without consideration of the absolute configuration of the pheromone. To determine the optical purity of the naturally occurring pheromone and to compare the biological activity of the enantiomers, it was necessary to devise syntheses that produced these enantiomers in high optical purity. (Scheme A for the (+) enantiomers, Scheme B for the (−) enantiomer).

Initial studies were directed towards the resolution of 2-methylpentanoic acid, which could be converted to 4-methyl-3-heptanone in good yield with two equivalents of ethyl lithium. To resolve 2-methylpentanoic acid into its optical isomers, α-methylbenzylamine seemed to be the best choice of resolving agent because both enantiomers of α-methylbenzylamine were commercially available in optically pure forms and both optically pure forms of 2-methylbutanoic acid, a homologue of 2-methylpentanoic acid, had been obtained by using the enantiomers of α-methylbenzylamine.[4] This approach, however, was abandoned because the diastereomeric salts had very similar solubility properties.

S-(+)-2-Methylpentanoic acid was synthesized by a modified version of a synthesis devised by Stallberg-Stenhagen.[5] Diethyl methylmalonate was alkylated with allyl chloride (63%), forming diethyl methylallylmalonate (**1**). The diester was saponified (81%) and the diacid (**2**) decarboxylated (98%) producing 2-methyl-4-pentenoic acid (**3**). This acid was resolved with quinine, giving S-(+)-2-methyl-4-pentenoic acid (**4**) which was hydrogenated over platinum oxide to S-(+)-2-methylpentanoic acid (**5**) (61%).

Levene and Marker obtained R-(−)-2-methylpentanoic acid (**6**) using quinine as the resolving agent and acetone as the solvent.[6] Attempts to duplicate these results led to a mixture slightly enriched in the (+) enantiomer. Changing the solvent system to a mixture of acetone and water led to the desired (−) enantiomer. The enantiomers of 4-methyl-3-heptanone (**7**, **8**) were synthesized from the optically active carboxylic acids by treatment with two equivalents of ethyl lithium in yields of approximately 78%.

There was no evidence that the reaction of ethyl lithium on the optically active acid precursors led to any racemization. When more than two equivalents of ethyl lithium were used, more of the major side product, 3-ethyl-4-methyl-3-heptanol was obtained.

Each synthetic enantiomer was bioassayed for its threshold level of activity on *Atta texana*. The (+) enantiomer is about 400 times more active than the (−) enantiomer. Since the enantiomers of 4-methyl-3-heptanone have identical physical and chemical properties in an achiral medium, it seems reasonable to postulate chiral sites on the receptor of the ant that fit the spacial disposition of only one of the enantiomers.

EXPERIMENTAL

General. M.ps were determined on a Kofler micro hot stage apparatus and are uncorrected. Mass spectra were obtained on a Hitachi RMU6 mass spectrometer, NMR spectra on a Varian A-60 NMR spectrometer, IR spectra on either Perkin-Elmer model 621 or 137 spectrometers, optical rotations on a Durren Jasco recording spectropolarimeter model ORD/UV/CD-5 with a cylindrical metal cell, demountable, with 10 mm fixed path length containing fused silica windows (0·5 ml volume). All GLC purifications were achieved with a Varian model 1740 gas chromatograph fitted with dual flame ionization detectors.

Starting materials. Diethyl methylmalonate, allyl chloride, (±)-2-methylpentanoic acid, (±)-4-methyl-3-

heptanone, sodium ethoxide, and the mono and dihydrates of quinine were obtained from Aldrich Chemical Company. Allyl chloride, (±)-2-methylpentanoic acid and pentane were distilled prior to use. Ethyl lithium was obtained from Ventron Corporation.

Isolation of S-(+)-4-methyl-3-heptanone from Atta texana. *Atta texana* heads (123 g) were extracted in a Waring blender with 4, 200 ml portions of pentane. The combined extract was dried (Na_2SO_4) and concentrated by fractional distillation (760 mm) and the residual orange oil (5 ml) was cooled overnight. The mixture was centrifuged to remove precipitated solids and the supernatant oil was subjected to preparative GLC. The component corresponding to S-(+)-4-methyl-3-heptanone was collected from 5% Hi Eff Diethyleneglycol Succinate (DEGS) on Chromosorb G 60/80 mesh, 3 m × 6·3 mm, He flow rate, 30 cm^3/min fitted with a precolumn packed with Chromosorb G 60/80, column temp 90°. The compound was further purified on 4% 1,2,3 tris-(2-cyano-ethoxy) propane (TCEP) on Chromosorb G 60/80 mesh, 7·3 m × 3 mm, He flow rate, 20 cm^3/min column temp 90°. The isolated ketone gave a single sharp peak when injected on the following capillary GLC columns at a He flow rate of 3 cm^3/min: Carbowax 20M, 30·5 m × 0·5 mm, column temp 80°; DEGS, 30·5 m × 0·5 mm, column temp 70°; Apiezon L, 15·2 m × 0·5 mm, column temp 87°. The natural ketone also gave a single peak when coinjected with an authentic sample of (±)-4-methyl-3-heptanone on all of the above capillary columns. The IR and mass spectra of the natural ketone were identical with the spectra of an authentic sample of (±)-4-methyl-3-heptanone. The natural ketone had $[\alpha]_D^{25} = +22{\cdot}1° \pm 0{\cdot}4°$ (*c*, 1·0, hexane).

Isolation of S-(+)-4-methyl-3-heptanone from Atta cephalotes. *Atta cephalotes* heads (262 grams) were extracted 5 times with a total of 920 ml of pentane. The combined extract was dried (Na_2SO_4) and concentrated by distillation (760 mm) to yield two to three ml of a yellow oil. The component corresponding to S-(+)-4-methyl-3-heptanone was purified by GLC on 5% Hi Eff DEGS on Chromosorb G 60/80 mesh, 3 m × 6·3 mm, He flow rate, 30 cm^3/min fitted with precolumn, column temp 95°, followed by chromatography on 4% carbowax 20 M on Chromosorb G 60/80 mesh, 7·3 m × 3 mm, He flow rate, 30 cm^3/min column temp 95°. The ketone showed a single peak on all three previously described capillary columns. Coinjection of the natural ketone with a sample of (±)-4-methyl-3-heptanone also gave a single peak on all three capillary columns. The IR and mass spectra of the natural ketone were identical with those of authentic sample of (±)-4-methyl-3-heptanone. The natural ketone had $[\alpha]_D^{27} = +22{\cdot}0 \pm 0{\cdot}4°$ (*c*, 1·0, hexane).

Diethyl methylallylmalonate (**1**). To a stirred soln of anhyd ether (25 ml) and EtOH (100 ml), NaOEt (17·0 g; 0·25 mol) was added. The soln was cooled to room temp, and 43·6 g (0·25 mol) of diethyl methylmalonate was added over a period of ½ h. After an additional ½ h at room temp, 19·1 g (0·25 mol) of allyl chloride was added, and the mixture was refluxed for 1 h. The mixture was cooled, filtered through Celite, concentrated, and distilled, to give 33·7 g (63%) of **1**: b.p. 103–108° (15 mm); IR (neat) 3·25 and 6·09 μm (C=C), 5·81 μm (ester C=O); NMR ($CDCl_3$) δ 5·68 (m, 1, $\underset{|}{H}-C=CH_2$), δ 5·10 (m, 2, $H-\underset{|}{C}=CH_2$), δ 4·21 (q, 4, $-CO_2-\underset{|}{CH_2}-CH_3$), δ 2·62 (d, 2, $-\underset{|}{CH_2}-CH=CH_2$), δ 1·40 (s, 3, $-\underset{|}{C}-CH_3$), δ 1·25 (t, 6, $-CO_2-CH_2-\underset{|}{CH_3}$). The above procedure was repeated on 56·3 g of diethyl methylmalonate yielding an additional 37·7 g of the diester. Lit.[5] b.p. 89·7–90·5°, 6·5–7 mm. (Found: C, 61·86; H, 8·59. Calc for $C_{11}H_{18}O_4$: C, 61·65; H, 8·48%).

Methylallylmalonic acid (**2**). To a stirred soln of **1** (55·0 g; 0·257 mol) was added dropwise 100 ml of 12M KOH (50 ml abs EtOH + 50 ml H_2O). After addition, the mixture was refluxed for 1 h and then acidified with 6M HCl in abs EtOH. The mixture was concentrated under reduced pressure, and the last traces of water were removed by azeotroping with anhyd benzene. The white solid residue was dissolved in boiling benzene, filtered hot, and allowed to crystallize on cooling. The crystals were collected and dried in a vacuum dessicator; yielding 32·9 g (81%) of **2**: m.p. 88–91°; IR ($CHCl_3$) 3·30 μm (broad, OH), 6·06 μm (C=C), 5·82 μm (acid C=O); NMR ($CDCl_3$), δ 11·95 (s, 2, —COOH), δ 5·83 (m, 1, $\underset{|}{H}-C=CH_2$), δ 5·18 (m, 2, $H-\underset{|}{C}=CH_2$), δ 2·70 (d, 2, $H_2C-\underset{|}{CH}=CH_2$), δ 1·47 (s, 3, $-\underset{|}{C}-CH_3$). (Found: C, 53·32; H, 6·12. Calc for $C_7H_{10}O_4$: C, 53·15; H, 6·39%).

2-Methyl-4-pentenoic acid (**3**). The diacid **2** (16·6 g, 0·105 mol) was vacuum distilled at 160° (20 mm); yield 11·7 g (98%) **3**: b.p. 92–94° (14 mm). IR (neat) 5·84 μm (acid C=O), 6·07 μm (C=C); NMR ($CDCl_3$) δ 11·85 (s, 1, —COOH), δ 5·80 (m, 1, $\underset{|}{H}-C=CH_2$), δ 5·04 (m, 2, $H-\underset{|}{C}=CH_2$), δ 2·38, (m, 3, $\underset{|}{H}-\underset{|}{C}-CH_2-\underset{|}{C}=CH_2$), δ 1·17 (d, 3, $-\underset{|}{C}-CH_3$). Mass spectrum *m/e* 114 (M^+), base peak 41 ($CH_2=CH-\dot{C}H_2$), 69 slightly less intense than base peak ($M^+ - COOH$). Lit.[5] b.p. 112·0–112·7°, 29 mm. (Found: C, 62·91; H, 8·79. Calc for $C_6H_{10}O_2$: C, 63·12; H, 8·85%).

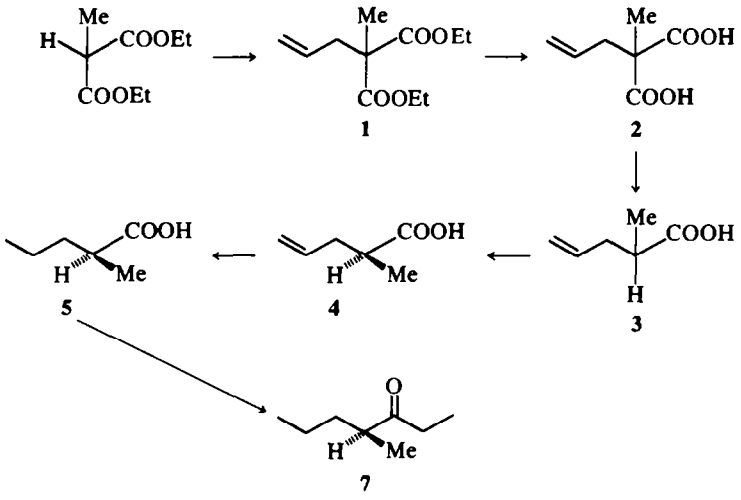
Resolution of 2-methyl-4-pentenoic acid (**4**). To 45·2 g (0·125 mol) of quinine dihydrate dissolved in 700 ml boiling acetone was added 14·2 g (0·125 mol) of 2-methyl-4-pentenoic acid. The soln was mixed rapidly for 5 min, cooled to room temp and allowed to crystallize overnight. The crystals were filtered and dried in a vacuum dessicator; yield 31·3 g of the quinine salt. The quinine salt was recrystallized 5 more times from acetone yielding 9·3 g of quinine salt. IR ($CHCl_3$) 3·10 μm (OH), 6·20 μm (COO^-); NMR ($CDCl_3$) δ 3·81 (s, 3, —OCH_3), δ 1·17 (d, 3, $H-\underset{|}{C}-CH_3$). The quinine salt was decomposed with 25 ml of 2 M HCl and extracted with four, 100 ml portions of ether. The ether extracts were combined, dried (Na_2SO_4), concentrated, and distilled; yield 2·1 g (7%) of S-(+)-2-methyl-4-pentenoic acid: $[\alpha]_D^{25} = +10{\cdot}5° \pm 0{\cdot}2°$ (*c*, 1·0, $CHCl_3$).* Lit.[5] $[\alpha]_D^{16} = +7{\cdot}62°$, neat.

S-(+)-2-methylpentanoic acid (**5**). To 29 mg

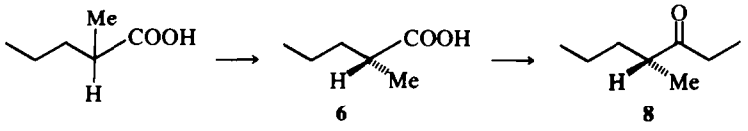
*The absolute configuration of 2-methyl-4-pentenoic acid has previously been established (Stina Ställberg-Stenhagen and Einar Stenhagen, *Arkiv Kemi, Min. Geol.* **24B**, 1 (1946)) thus establishing the absolute configuration of the natural pheromone and its enantiomer.

(0·128 mmol) of Adam's platinum oxide catalyst suspended in 25 ml of abs EtOH was added 1·0 g (8·77 mmol) of **4**. The purged soln was under H_2 at one atmosphere for 45 min (volume of H_2 consumed, 206 ml calc for 1·0 g of acid, 196 ml). The soln was filtered through Celite, concentrated, and distilled; yield 620 mg (61%) of **5**: b.p. 100–101° (19 mm); IR (CCl_4) 5·91 μm (acid C=O); NMR ($CDCl_3$) δ 11·55 (s, 1, —COOH), δ 2·45 (m, 1, methine hydrogen), δ 1·20 (d, 3, HC—CH_3); mass spectrum; *m/e* 116 (M^+), 74, base peak (McLafferty rearrangement); $[\alpha]_D^{27} = +15·5° \pm 0·3°$ (*c*, 1·0, $CHCl_3$). Lit[5] b.p. 100°, 7 mm; $[\alpha]_D^{16} = +17·15°$, neat. (Found: C, 62·03; H, 10·32. Calc for $C_6H_{12}O_2$: C, 62·02; H, 10·43%).

Resolution of 2-methylpentanoic acid (**6**). Quinine monohydrate (42·5 g, 0·124 mol) was added slowly to a stirred soln of 2-methylpentanoic acid (14·4 g; 0·124 mol) in 100 ml boiling acetone. Distilled water was added until the soln became turbid. The soln was cooled to room temp and a seed crystal of the quinine salt was added ($[\alpha]_D^{27} = -9·5° \pm 0·2°$ (*c*, 1·0, $CHCl_3$) on free acid)* and the salt



SCHEME A



SCHEME B

S-(+)-4-methyl-3-heptanone (**7**). To a rapidly stirred soln of S-(+)-2-methylpentanoic acid (86·5 mg; 0·75 mmol) in 4 ml of dry ether was added 1·17 ml (1·5 mmol) of EtLi (1·28 M in benzene) in 3 ml dry ether. Following addition, two additional milliliters of ether were added, and the mixture was refluxed for 30 min. The soln was hydrolyzed by dropwise addition to a rapidly stirring soln of ice water. The mixture was extracted with ether and dried (Na_2SO_4), and its volume was reduced to 2 ml by distillation. A chromatogram of the soln on the 4% TCEP column previously described showed two peaks; the major peak (78%) corresponding to S-(+)-4-methyl-3-heptanone and the minor peak (22%) corresponding to 3-ethyl-4-methyl-3-heptanol. Samples of the ketone were collected by preparative GLC for biological testing. The IR and mass spectra were identical with the spectra of an authentic sample of (±)-4-methyl-3-heptanone. The optical rotation showed $[\alpha]_D^{27} = +21·0° \pm 0·4°$ (*c*, 1·0, hexane). (Found: C, 74·91; H, 12·33. Calc for $C_8H_{16}O$: C, 74·92; H, 12·60%).

R-(−)-4-methyl-3-heptanone (**8**). To a stirred soln of R-(−)-2-methylpentanoic acid (150·6 mg; 1·3 mmol) in 5 ml dry ether was added 2·1 ml (2·7 mmol) of EtLi (1·28 M in benzene) in 3 ml dry ether. Following addition, the system was refluxed for 30 min, cooled and hydrolyzed by adding the ether soln dropwise to a rapidly stirring soln of ice water. The ether layer was separated, dried (Na_2SO_4) and concentrated to a volume of 2 ml. Samples of the ketone were collected by preparative GLC for biological testing. The IR and mass spectra were identical with the spectra

*The partially resolved quinine salt seed crystals were obtained by recrystallization of a smaller quantity of quinine salt from a 50/50, V/V, water–acetone solvent system. Six recrystallizations from this solvent system gave a free acid with the above rotation.

allowed to crystallize out overnight. The quinine salt was recrystallized 12 more times, from acetone–water, with the last 5 recrystallizations taking place without seeding. The quinine salt was decomposed using 3M HCl, the free acid extracted with ether and dried (Na_2SO_4). The mixture was concentrated and distilled giving 1·2 g (8·3%) of R-(−)-2-methylpentanoic acid: b.p. 88–89° (10 mm); $[\alpha]_D^{25} = -15·7° \pm 0·3°$ (*c*, 1·0, $CHCl_3$). Lit[6] b.p. 96°, 15 mm; $[\alpha]_D^{25} = -18·4°$.

of an authentic sample of (±) 4-methyl-3-heptanone. The optical rotation showed $[\alpha]_D^{25} = -21{\cdot}5° \pm 0{\cdot}4°$ (*c*, 1·0, hexane). (Found: C, 75·21; H, 12·68. Calc for $C_8H_{16}O$: C, 74·02; H, 12·60%).

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