Phytochemistry, 1977, Vol. 16. pp. 263–264, Pergamon Press, Printed in England.

HYDROCARBONS FROM *CIRSIUM JAPONICUM*

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(*Revised received* 29 *July* 1976)

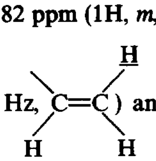
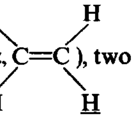
Key Word Index—*Cirsium japonicum*; Compositae; root oil; hydrocarbons; dihydro- and tetrahydro-aplotaxene.

Abstract—Aplotaxene, dihydro-, tetrahydro- and hexahydro- aplotaxene, 1-pentadecene, cyperene, caryophyllene, thujopsene and α-himachalene were found in the root oil of *Cirsium japonicum*. The isolation of dihydro- and tetrahydro-aplotaxene is the first report from natural sources. The latter is a biogenetically important intermediate from oleic acid to C_{17}-acetylene compounds.

INTRODUCTION

It has been suggested that a biogenetic pathway to acetylenic compounds from oleic acid probably exists [1] but no intermediates have been isolated from natural sources. In the work reported here, one such intermediate was isolated from the root oil of *Cirsium japonicum* (Noazami).

RESULTS AND DISCUSSION

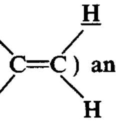
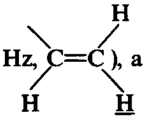
Dihydroaplotaxene (1), $C_{17}H_{30}$, *m/e* 234 (M^+), showed no absorption maximum in its UV spectrum but on hydrogenation consumed 3 molar equivalents of hydrogen, and gave *n*-haptadecane. Thus, this compound is *n*-heptadecatriene. The IR bands at 3075 (CH_2), 1640 (C=C), 992 (CH) and 909 (CH_2) cm^{-1} showed the presence of a vinyl group. Although a band characteric of a disubstituted ethylenic double bond was observed at 3010 cm^{-1}, a *trans*-disubstituted absorption was absent in the region 980–960 cm^{-1} but a *cis*-disubstituted absorption was observed at 721 cm^{-1} which overlapped with the —CH_2— rocking vibration. The PMR spectrum provided additional information about the structure of (1); a vinyl group at 5.82 ppm (1H, *m*, $C\underline{H}{=}CH_2$), 4.97 ppm (1H, *d–m*, J = 17 Hz,  $\begin{matrix} & & \underline{H} \\ & C{=}C & \\ H & & H \end{matrix}$) and 4.92 ppm (1H, *d–m*, *J* = 10 Hz,  $\begin{matrix} & & H \\ & C{=}C & \\ H & & \underline{H} \end{matrix}$), two *cis*-disubstituted ethylenic double bonds at 5.34 ppm (4H, *m*, *J* < 11 Hz, $[C\underline{H}{=}C\underline{H}]_2$), a —$CH_2$— group situated between two double bonds at 2.75 ppm (2H, *m*, $CH{=}CH{-}C\underline{H}_2{-}CH{=}CH$), three —$CH_2$— groups connected to a double bond at 2.04 ppm (6H, *m*, $[C{=}C{-}C\underline{H}_2]_3$), a Me group at 0.86 ppm (3H, *t*, *J* = 6 Hz, $CH_2{-}C\underline{H}_3$) and six CH_2 groups at 1.33 ppm (12H, *s–m*, $[C\underline{H}_2]_6$). *n*-Heptadecatriene after ozonlysis, gave formaldehyde, caproic acid and pimelic acid.

From the chemical and physical data, the hydrocarbon could have two possible structure **1** or **2** but as the —CH_2— protons connected to a double bond at 2.04 ppm had been decoupled and the vinyl proton at 5.82 ppm ($C\underline{H}{=}CH_2$) changed from multiplet signals to typical quartet signals the compound had a partial structure $(CH_2)_5{-}CH{=}CH_2$ and was thus 1, 8, 11-heptadecatriene (**1**).

$Me{-}(CH_2)_4{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}(CH_2)_5{-}CH{=}CH_2$ **1**

$Me{-}(CH_2)_4{-}CH{=}CH{-}(CH_2)_5{-}CH{=}CH{-}CH_2{-}CH{=}CH_2$ **2**

$Me{-}(CH_2)_7{-}CH{=}CH{-}(CH_2)_5{-}CH{=}CH_2$ **3**

Tetrahydroaplotaxene (3), $C_{17}H_{32}$, *m/e* 236 (M^+), also had no UV absorption maximum. On hydrogenation the hydrocarbon consumed 2 molar equivalents of hydrogen and gave *n*-heptadecane. This compound is thus *n*-heptadecadiene. The IR bands, at 3075 (CH_2) 1640 (C=C), 995 (CH), 910 (CH_2) cm^{-1}, indicated the presence of a vinyl group and those at 3005 and 722 cm^{-1} a *cis* disubstituted ethylenic double bond. The PMR spectrum showed signals at 5.82 ppm (1H, *m*, $C\underline{H}{=}CH_2$), 4.97 ppm (1H, *d–m*, *J* = 17 Hz,  $\begin{matrix} & & \underline{H} \\ & C{=}C & \\ H & & H \end{matrix}$) and 4.92 ppm (1H, *d–m*, *J* = 10 Hz,  $\begin{matrix} & & H \\ & C{=}C & \\ H & & \underline{H} \end{matrix}$), a *cis*-disubstituted ethylenic double bond at 5.34 ppm (2H, *m*, *J* < 10 Hz, $C\underline{H}{=}C\underline{H}$), 3 —$CH_2$— groups connected to a double bond at 2.00 ppm (6H, *m*, $[C{=}C{-}CH_2]_3$), a Me group at 0.86 ppm

(3H, *t*, $J = 6$ Hz, $CH_2—C\underline{H}_3$) and 9 CH_2 groups at 1.24 ppm (18 H, *s–m*, $[C\underline{H}_2]_9$).

n-Heptadecadiene was ozonized, and gave HCHO pelargonic acid and pimelic acid. Thus, the structure of tetrahydroaplotaxene was found to be 1,8-heptadecadiene.

The isolation of dihydro- and tetrahydro-aplotaxene is the first report from natural sources. The latter is biogenetically important intermediate from oleic acid to C_{17}-acetylene compounds [1].

Aplotaxene [2], hexahydroaplotaxene (1-heptadecene), 1-pentadecene, cyperene, caryophyllene, thujopsene and α-himachalene were also identified as constituents of the root oil.

EXPERIMENTAL

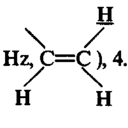
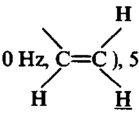
UV spectra were measured in EtOH. IR spectra were recorded as liquid films and KBr disks. NMR spectra were determined using TMS as an internal standard in $CDCl_3$. To analyse volatile constituents and to identify the minor components, GLC was carried out using 25% PEG 6000 (He 30 ml/min, 165°) and 5% SE 30 (He 15 ml/min, 165°).

Extraction and separation. Fresh roots (6.3 kg) of *C. japonicum*, collected in the beginning of May at Fukuoka prefecture, were chopped finely and extracted with Et_2O (7.6 l.) for 2 weeks at room temp. The oil (43 g, 0.68% of the root) was obtained by evaporating the Et_2O under red pres. The extract (43 g) was chromatographed on a deactivated Al_2O_3 column. *n*-Hexane eluted hydrocarbons (79% of the extract), C_6H_6–EtOAc (4:1) and EtOAc eluted polar components (18% of the extract). The hydrocarbon (34 g) was rechromatographed on a deactivated Al_2O_3 column with *n*-hexane and divided into 7 fractions.

Dihydroaplotaxene (**1**). Fraction 6 (11% of hydrocarbon) found: C, 87.30; H, 12.67%. Calcd for $C_{17}H_{30}$: C, 87.10; H, 12.90%. **1** (49 mg) was hydrogenated over Adams PtO_2 (13 mg) in HOAc and absorbed 3.1 mol of H_2. *n*-Heptadecane (23.2 mg) was obtained, and identified by MS and GLC comparison with authentic material. **1** (111 mg) was ozonised in HOAc (15 ml) at 0° for 1 h. The reaction mixture was diluted with H_2O (30 ml) and refluxed for 1 h. The mixture was distilled into an acidified alcoholic soln acidified of 2,4-DNPH and the resultant soln chromatographed on Si gel TLC with hexane–C_6H_6–EtOAc (7:2:1). A spot (R_f 0.18) was identified as HCHO 2,4-DNPH by comparison with an authentic sample. The distilled residue of the reaction mixture was extracted with 100 ml of Et_2O–H_2O (1:1). From the aq layer, an oily substance (25 mg) was isolated, and recrystallized from C_6H_6 mp 100–102°C. IR spectrum, 1695, 1430, 1410, 1350, 1275, 1202, 920, 732, 690 cm^{-1}, was identical with that of pimelic acid. From the Et_2O layer, an oil (trace) was obtained which by TLC on Si gel with *iso* PrOH–HOAc (98.5:1.5), gave a spot (R_f 0.62) identical to that of authentic caproic acid. MS of **1** showed peaks at *m/e* 41 (100%) 55 (61), 67 (91), 81 (71), 95 (38), 110 (39), 234 (14, M^+).

Tetrahydroaplotaxene (**3**). Fraction 5 (7%) found: C, 86.65; H, 13.44%. Calcd for $C_{17}H_{32}$: C, 86.36; H, 13.64%. **3** (58 mg) was hydrogenated over Adams PtO_2 (15 mg) in HOAc and absorbed 2.2 mol of H_2. The product (32 mg) was identified as *n*-heptadecane by MS and GLC comparison with an authentic sample. **3** (153.8 mg) was ozonized in HOAc (20 ml) at 0° for 1 h. HCHO and pimelic acid were identified as reaction products using similar methods to those used for (**1**). The third product was identified as pelarganic acid by IR, MS and TLC comparison with authentic material. MS spectrum of **3** showed the fragment peaks at *m/e* 29 (43%), 41 (99), 43 (100), 54 (47), 55 (77), 67 (56), 81 (49), 82 (52), 96 (52), 236 (20, M^+).

Aplotaxene. Fraction 7 (67%) found: C, 87.62; H, 12.03%. Calcd for $C_{17}H_{28}$; C, 87.86; H, 12.14%. $[\alpha]_D^{25}$ 0°. The hydrocarbon (98.6 mg) was hydrogenated over Adams PtO_2 (24.7 mg) in HOAc. The uptake of H_2 corresponded to 4.1 mol. The product (71 mg) was isolated as crystals mp 20.5–21.5°, Found: C, 84.96; H, 14.90%. Calcd for $C_{17}H_{36}$: C, 84.91; H, 15.09%. The MS was identical to that of *n*-heptadecane. Ozonolysis of *n*-heptadecatetraene gave HCHO and propionaldehyde (TLC-2,4-DNPH) and pimelic acid.

The MS of aplotaxene showed peaks at *m/e* 41 (32%), 55 (44), 67 (70), 79 (100), 80 (50), 91 (35), 93 (45), 95 (37), 108 (65), 232 (5, M^+). PMR (ppm): 5.82 (1H, *m*, $C\underline{H}{=}CH_2$), 4.97 (1H, *d–m*, $J = 17$ Hz,  $\underset{H}{\overset{\diagdown}{C}}{=}\underset{H}{\overset{\underline{H}}{C}}$), 4.92 (1H, *d–m*, $J = 10$ Hz,  $\underset{H}{\overset{\diagdown}{C}}{=}\underset{\underline{H}}{\overset{H}{C}}$), 5.35 (6H, *m*, $J < 11$ Hz, *cis* $[C\underline{H}{=}C\underline{H}]_3$, 2.78 (4H, *t–m*, J = 6 *Hz*, $CH{=}CH—C\underline{H}_2—CH{=}CH—C\underline{H}_2—CH{=}CH$), 2.04 (6H, *m*, $[C{=}C—C\underline{H}_2]_3$), 0.96 (3H, *t*, $J = 6$ Hz, $CH_2—C\underline{H}_3$) 1.33 (6H, *s–m*, $[CH_2]_3$). The CH_2 protons at 2.04 ppm had been decoupled. A vinyl proton at 5.82 ppm ($C\underline{H}{=}CH_2$) changed from complex signals to typical quartet signals. ^{13}C—NMR spectrum 14.0, 20.3, 114.1, 139.0 ppm, showed the presence of an Et group connected to a double bond and a vinyl group. From these chemical and physical data, the hydrocarbon had a partial structure of $(CH_2)_5—CH{=}CH_2$ group, and was confirmed as 1, 8, 11, 14-heptadecatetraene. IR spectrum, 3075, 3015, 1640, 992, 910, 715 cm^{-1}, was identical with that of aplotaxene [2].

Hexahydroplotaxene ((*1-heptadecene*). Fraction 3 contained hyxahydroaplotaxene (2%) which was identified by GLC and MS comparison with an authentic sample.

1-Pentadecene. Fraction 2 (9%) was 1-pentadecene which was identified by IR, GLC and MS comparison with an authentic sample.

Cyperene. Fraction 1 (3%) was identified as cyperene. PMR (ppm): 0.74 (6H, *s*, $C[C\underline{H}_3]_2$, 0.77 (3H, *d*, $J = 6$ Hz, $CH{=}C\underline{H}_3$) 1.60 (3H, *d*, J = 1.5 Hz, $CH{=}C—C\underline{H}_3$). MS: *m/e* 41 (52%), 55 (37), 91 (31), 105 (39), 119 (39), 161 (41), 189 (59), 204 (100, M^+). IR spectrum identical to lit. [3].

Caryophyllene, thujopsene and α-himachalene. These compounds were identified as caryophyllene (trace, PEG 6000; t_R 6.3 min, SE 30; 5.4), thujopsene (trace, 7.2, 5.4) and α-himachalene (trace, 7.9, 5.6) by GLC comparison with authentic samples.

Acknowledgements—The author thanks Prof S. Hayashi for measurement of MS, and Y. Tsuno for measurement of ^{13}C-NMR spectrum.

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