TETRAHYDRORHOMBIFOLINE, A FURTHER CONSTITUENT OF *LUPINUS OSCAR-HAUGHTII* AND *L. TRUNCATUS*

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Gas chromatography/mass spectrometry (gc/ms) has become a useful analytical tool for the rapid identification of quinolizidine alkaloids in species of the genus *Lupinus*, since diastereomeric pairs can usually be resolved (1-6). However, not all solute components in lupine extracts studied by gc/ms may be readily identifiable (2, 4, 6), and, in one such study, we were initially unable to identify a minor quinolizidine alkaloid constituent occurring in extracts of both *L. oscar-haughtii* and *L. truncatus* (6), as no molecular ion peak was apparent in the mass spectrum of this compound.

In the present work, this previously unassigned compound was isolated from *L. oscar-haughtii* and tentatively characterized as (+)-tetrahydrorhombifoline, by comparison of the physical data and pmr spectrum of the isolate with literature data (7). Since the detailed mass spectrum of this compound has not been published, we confirmed its identity by direct comparison (gc/ms, tlc) with an authentic sample synthesized from angustifoline using a modified Eschweiler-Clarke reagent (8). Tetrahydrorhombifoline was detected in *L. truncatus* (stems, roots, leaves and flowers) by gc/ms and tlc only, since insufficient material was available to isolate this compound and perform physical and spectroscopic measurements.

Tetrahydrorhombifoline has been postulated as an intermediate in biosynthetic pathways from lupanine to cytisine (9) and from lupanine to *N*-methyltetrahydrocytisine (10). Neither cytisine nor *N*-methyltetrahydrocytisine are known to accumulate in the genus *Lupinus*, and tetrahydrorhombifoline appears to be rare in the lupines (11). We have detected this compound only in *L. oscar-haughtii* and *L. truncatus* among over 25 lupines investigated. It may be pointed out that based on the relative abundance of its mass spectral fragment ions, the unidentified alkaloid A of Wink and coworkers, observed recently in a *L. polyphyllus* cell suspension culture (4), is probably tetrahydrorhombifoline, a known constituent of the seeds of this species (12).

In addition, we would like to draw attention to the possibility of confusion of tetrahydrorhombifoline with its structural isomer, *N*-methylangustifoline, during gc/ms analysis. The latter compound, recently claimed to be a constituent of *L. polyphyllus* (13), was prepared from angustifoline by a literature method (8). Both alkaloids possessed the same retention time on the glc stationary phase used and exhibited identical mass spectral fragment peaks that varied only in relative abundance, although the compounds were separable by tlc. This example demonstrates the need to substantiate gc/ms screening data of lupine quinolizidine alkaloids by tlc to prevent errors in compound identification.

EXPERIMENTAL[1]

PLANT MATERIAL.—Lyophilized 80% ethanolic extracts of the stems (10 g) and the inflorescences (in fruit, 10 g) of *Lupinus oscar-haughtii* C. P. Smith (Leguminosae), collected in Colombia in March, 1972, and of the combined stems, roots, leaves and flowers of *L. truncatus* Nutt. ex Hook. & Arn. (Leguminosae) (3 g), collected in California in March, 1965, were supplied through the Developmental Therapeutics Program (Natural Products Branch) of the National Cancer Institute, formerly the Cancer Chemotherapy National Service Center, Bethesda, Maryland. Voucher specimens representing these collections are deposited at the Herbarium of the National Arboretum, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C.

EXTRACTION OF ALKALOIDS AND CHROMATOGRAPHIC SCREENING METHODS.—Crude alkaloidal extracts of the two *L. oscar-haughtii* plant parts, and of *L. truncatus*, were prepared from the original ethanolic extracts as described previously (5, 6). These were then subjected to gc/ms on 3% OV-17 on Gas Chrom Q (100–120), with the temperature programmed 180–310°, 4° min^{-1}, and the flow rate of helium 18 ml min^{-1}. The injector temperature was set at 260°, the interface separator temperature at 240°, and spectra were recorded with 6 sec between scans. The alkaloidal extracts were chromatographed by tlc on silica gel GHLF[2] in S_1, chloroform-methanol-ammonium hydroxide solution (85:15:1), S_2, methanol-ammonium hydroxide solution (19:1) and S_3, diethyl ether-methanol-ammonium hydroxide solution (100:10:1).

ISOLATION OF TETRAHYDRORHOMBIFOLINE.—Tetrahydrorhombifoline was isolated by preparative tlc of the crude alkaloidal extracts of both the stems and the fruiting inflorescences of *L. oscar-haughtii* on silica gel GHLF[2] in S_1 (R_f, 0.82) and S_2 (R_f, 0.57). This compound, which was obtained as an oil, exhibited the following data: $[\alpha]^{26}_{365}$+260°, $[\alpha]^{26}_{436}$+150°, $[\alpha]^{26}_{546}$+83°, $[\alpha]^{26}_{578}$+77° and $[\alpha]^{26}_{589}$+70° (c 0.03, ethanol); ir (ν max) (KBr) 3170, 2850, 2760, 1625, 995 and 905 cm^{-1}; pmr ($CDCl_3$) δ 1.10–3.20 (19H, m), 3.46 (1H, m, H-6), 4.70 (1H, bd, J=14 Hz, H-10 eq.), 4.83, 5.08 (2H, m, C=CH_2, H_2-17) and 5.70 (1H, bm, H-16); ms, m/z (20 eV) 248 (M^+, 2%), 208 (14), 207 (100), 136 (1), 112 (20), 108 (11), 94 (3), 83 (5), 59 (3) and 58 (87); gc/ms, Rt, 12.7 min, ms, m/z M^+ missing, 208 (13), 207 (94), 112 (19), 108 (10), 94 (5), 58 (100), 55 (15), 42 (13) and 41 (18); tlc, S_1, R_f 0.82, S_2, R_f 0.57 and S_3, R_f 0.74.

PREPARATION OF TETRAHYDRORHOMBIFOLINE AND *N*-METHYLANGUSTIFOLINE FROM ANGUSTIFOLINE.—Angustifoline (3 mg) was obtained as previously described (6) and divided into two portions. Treatment of angustifoline with formaldehyde and sodium borohydride in aqueous methanol under published reaction conditions (8) resulted in the formation of tetrahydrorhombifoline. Methyl iodide in acetone was used to synthesize *N*-methylangustifoline from angustifoline (8). These reaction products were purified by preparative tlc in S_1 (R_f, 0.82, tetrahydrorhombifoline, 1 mg) and S_3 (R_f, 0.68, *N*-methylangustifoline, 1 mg). The tetrahydrorhombifoline so produced was identical to the isolate from *L. oscar-haughtii* by gc/ms and tlc in S_1-S_3. *N*-Methylangustifoline exhibited the following data: gc/ms, Rt, 12.7 min, ms, m/z M^+ missing, 208 (14), 207 (100), 112 (45), 108 (8), 94 (7), 58 (26), 55 (14), 42 (12) and 41 (18); tlc, S_1, R_f 0.82, S_2, R_f 0.48, S_3, R_f 0.68.

[1]Optical rotations were obtained on a Perkin-Elmer 241 polarimeter, and the ir spectrum was measured on a Perkin-Elmer 710 instrument. The pmr spectrum was recorded on a Varian T-60A instrument, operating at 60 MHz, with a Nicolet model TT-7 Fourier Transform attachment. Tetramethylsilane was used as the internal standard, and chemical shifts are reported in δ (ppm) units. Combined gc/ms (70 eV) was performed on a Varian MAT 112S mass spectrometer, equipped with a Varian 166 data system, linked to a Varian 1400 model gas chromatograph. The low resolution mass spectrum was also recorded on this mass spectrometer.

[2]Analtech, Inc., Newark, Delaware.

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