mg, 1.56×10^8 dpm, New England Nuclear) dissolved in water was fed by the wick method to 40 3-month-old *N. glutinosa* plants growing in soil in a greenhouse (June). After 7 days the plants (fresh wt 2.8 kg) were harvested (residual activity not absorbed by the plants: 0.07%), macerated with chloroform and concentrated NH_3, and worked up as previously described.[29] The crude alkaloids (1.76×10^6 dpm, 1.1% incorporation) were separated by TLC,[29] affording nornicotine (187 mg), crystallized to constant activity as its dipicrate (1.08×10^5 dpm/mmol), and nicotine (472 mg), assayed as its diperchlorate (3.12×10^5 dpm/mmol). The anabasine (3.1 mg) and anatabine (16.6 mg) purified as their dipicrates had negligible activity ($<10^3$ dpm/mmol).

Registry No.—*RS*-**1**, 616-07-9; **2**, 54-11-5; **2** diperchlorate, 59888-66-3; **3**, 494-97-3; **3** dipicrate, 6255-01-2; **4**, 36740-09-7; **5**, 486-56-6; **5** dipicrate, 59888-69-6; **8**, 49835-54-3; **9**, 59888-67-4; **9** diperchlorate, 59888-68-5; **9** dipicrate, 59951-82-5; **10**, 59951-83-6; **10** dipicrate, 59980-68-6; **11**, 59888-70-9; nicotinic acid, 59-67-6; pyridine picrate, 3480-66-8; barium carbonate, 513-77-9; benzoic acid, 65-85-0; CO_2, 124-38-9; phenyl vinyl ketone, 768-03-6; *cis*-(*RS*)-5′-phenylnicotine, 59951-84-7; *cis*-(*RS*)-5′-phenylnicotine dipicrate, 59951-85-8; benzylamine, 100-46-9.

References and Notes

(1) Contribution No. 143 from this laboratory. This investigation was supported by Research Grant GM-13246 from the National Institutes of Health, U.S. Public Health Service, and also by Grant 929 from The Council for Tobacco Research—U.S.A., Inc. Presented in part at the 172nd National Meeting of the American Chemical Society, San Francisco, Calif., Sept 1976.
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Aphylline, Epiaphylline, 10,17-Dioxosparteine, Gramine, and Other Unexpected Alkaloids from *Lupinus hartwegii*

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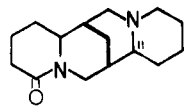
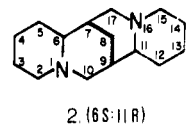
In addition to lupanine (1) and 13-hydroxylupanine, originally reported in flowering plants of *L. hartwegii*, four oxosparteines, aphylline (3), epiaphylline, 13-hydroxyaphylline (virgiline), and 10,17-dioxosparteine, not previously reported in any *Lupinus* species were isolated. 4-hydroxylupanine (nuttaline), α-isolupanine (6), and gramine (7) were also present in addition to five other partially characterized alkaloids. Even though carefully looked for, no sparteines **2**, **5**, **8**, lupinine, or angustifoline were detected.

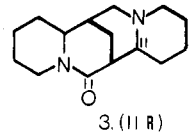
Lupanine (1), the major alkaloid reported in *Lupinus hartwegii*,[1] is viewed as being an oxidation product of sparteine (**2**).[2-4] However, this plant apparently forms little or no sparteine, but has been genetically crossed with *L. arboreus*, whose alkaloid is sparteine, to produce hybrids whichcan form both **1** and **2**.[5]



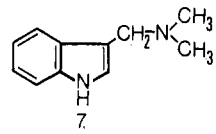
1. (11R)
6. (11S)



2. (6S:11R)
5. (6S:11S)
8. (6R:11R)



3. (11R)
4. (11S)



7

This apparent genetic separability of the ability to form 1 or 2 is consistent with our conclusion from $^{14}CO_2$ pulse labeling studies on three other *Lupinus* species, that sparteine and lupanine may be synthesized independently of one another.[6] These and other $^{14}CO_2$ studies[7] also revealed the presence of several kinetically interesting (rapidly turning over) components in the alkaloid extracts, one of which (unknown A) appeared to be present in *L. hartwegii* in significant amounts.

Therefore, as part of a study on the biosynthesis of lupanine from $^{14}CO_2$,[8] an investigation was undertaken of the alkaloids in this plant using freshly harvested material to (1) determine whether any sparteine was present; (2) confirm lupanine as the major alkaloid; (3) determine the structure of A; and (4) characterize as many other alkaloids as possible.

Results and Discussion

An examination of the basic extract of fresh 6 week old plants of *Lupinus hartwegii* by TLC indicated the presence of at least ten alkaloids. By the sequential use of TLC, GLC, and mass spectrometry 14 alkaloids were purified and characterized. The presence of lupanine and 13-hydroxylupanine (alkaloids C and E, respectively), the only alkaloids previously reported in this plant,[1] was confirmed although the latter was present in very small amounts (Table I). Though carefully looked for there was no trace of sparteine or the bicyclic base lupinine, which is consistent with our recent demonstration that this species cannot form either alkaloid at this stage of development when lupanine formation from $^{14}CO_2$ is very active.[8]

Alkaloids A1 and a2, both having the composition $C_{15}H_{24}NO$, gave mass spectra very similar to those reported for the HBr salts of the 10-oxosparteines[9] but markedly different from that of 17-oxosparteine.[10] The presence of M − CO fragments in the mass spectra of both, characteristic of 10 and 17-oxosparteines,[9] was confirmed by high-resolution mass spectrometry. A1 was unchanged under mild catalytic hydrogenation conditions contrary to that reported for aphylline (**3**)[11] but was completely converted to α-isosparteine (**5**) under more drastic conditions, confirming it to be epiaphylline (**4**). Alkaloid A2, on the other hand, was completely reduced under mild conditions to sparteine, confirming it to be aphylline.[11]

Alkaloid A3 gave a MS with an apparent M^+ at *m/e* 246 but no M − CO fragment, clearly different from that reported for 5,6-dehydrolupanine[12] but similar to that of 11,12-dehydrolupanine.[10] The instability of this substance to air is also typical of 11,12- and other dehydrolupanines[13,14] and further work on this substance when available would be of interest, since only one fully characterized dehydrolupanine has been reported to date while at least 12 such compounds are possible and implicated as possible intermediates in the biosynthesis of this group of alkaloids.[7]

GLC of zone B revealed in addition to α-isolupanine (**6**, B1) two additional components. The MS of B2 was unlike that reported for any quinolizidine alkaloid, with an apparent M^+ at *m/e* 264 and no M − CO fragment, suggestive of a hydroxylupanine but clearly different from that of 4-hydroxylupanine (D3) or 13-hydroxylupanine (E), both also found in this plant. An unusual M − 41 fragment confirmed by a metastable at *m/e* 188.4 was prominent. Again insufficient sample prevented further analysis.

Alkaloid B3 gave a weak iodoplatinate reaction observed for 17-oxolupanine[15] but was distinguishable from the latter by GLC (Table I) and by MS.[10] A MS identical with that reported for 10,17-dioxosparteine[9] and reduction of B3 to 17-oxosparteine, as would be expected if the bridgehead hydrogen at C-6 were cis to the methylene bridge,[16] confirmed its structure.

Zone D yielded five components on GLC, the major one proving to be identical with nuttaline (4-hydroxylupanine). This is the first report of its occurrence since it was first isolated from *L. nuttallii*.[17] The MS of D2 with an apparent M^+ at *m/e* 264 differed from that reported for any lupin alkaloid or alkaloid B2. The presence of apparent M − H_2O and M − CO fragments at *m/e* 246 and 236, respectively, suggested that it might be a hydroxyaphylline, which was confirmed by comparison with an authentic sample of virgilline (13-hydroxyaphylline). This is the first report of this alkaloid in any other species since its original isolation from *Virgilia orboides*.[18]

The three remaining alkaloids of this group, D3, D4, and D5, all had the longest retention times on GLC of any of those isolated but less than that of *O*-(2-pyrrolylcarbonyl)virgiline (Table I). They also had unusually low R_f's in TLC (system B). Only D5 was present in sufficient amounts to permit any further examination. The MS of D5 revealed an apparent M^+ at *m/e* 378. An extremely large metastable at *m/e* 204.5 confirmed the M − 100 transition to *m/e* 278 (base peak) while

Table I. Chromatographic Properties of Standard Alkaloids and Those of 6 Week Old *Lupinus hartwegii*

Compd[b]	Gas–liquid chromatography[a] t_R, min	Alkaloid[c]	% [d]	R_f on TLC system A	B	C
α-Isosparteine	3.5			0.07	0.18	0.44
Sparteine	4.3			0.11	0.62	0.46
β-Isosparteine	5.8			0.08	0.44	0.43
Gramine	6.5[e]	E1	18.0	0.16	0.60	0.73
17-Oxosparteine	24.5			0.87	0.86	0.82
Epiaphylline	25.5	A1	6.0	0.88	0.91	0.70
α-Isolupanine	27.5	B1	1.3	0.80	0.85	0.52
Lupanine	28.5	C1	54.1	0.72	0.85	0.52
Unidentified	29.5	B2	0.5	0.78	0.88	0.49
Aphylline	30.0	A2	4.0	0.85	0.90	0.61
Nuttalline	30.5	D1	9.0	0.58	0.08	0.41
Unidentified	30.7	A3	0.2	0.85		
13-Hydroxylupanine	39.5	E2	0.2	0.35	0.44	0.35
10,17-Dioxosparteine	40.5	B3	0.3	0.82		
Virgiline	41.0	D2	1.1	0.60	0.52	0.45
17-Oxolupanine	43.0			0.84	0.85	0.88
Unidentified	52.5	D3	0.1	0.44	0.05	
Unidentified	54.5	D4	0.2	0.46	0.05	
Unidentified	68.5	D5	5.0	0.50	0.07	0.56
O-(2-Pyrrolylcarbonyl)virgiline	110.0[f]			0.86	0.94	0.76

[a] Ten percent QF-1 column; see text for conditions. [b] Arranged in order of increasing retention times on GLC. [c] Letter and number designations explained in text. [d] Percent of total plant alkaloids calculated from GLC peak areas and the relative weights of the alkaloids eluted from TLC. [e] Most of the injected alkaloid decomposed on GLC. [f] 250 °C isothermal.

M − 17 losses from both the *m/e* 378 and 278 peaks were confirmed by metastables at *m/e* 343 and 245. Though the compound was suspected of being an ester of a hydroxylupanine, no alkaloid could be recovered from even mild alkaline hydrolysis generally employed to characterize a number of esters of virgiline and 13-hydroxylupanine.[18,19] This apparent instability of the parent alkaloid under alkaline conditions is reminiscent of 5,6-dehydrolupanine[7] and would appear to rule out 13-hydroxylupanine or virgiline as the parent alkaloid. Further speculation would be fruitless in the absence of additional data.

The decomposition of the major alkaloid from zone F during GLC as well as its low R_f on TLC (system A) suggested the presence of a highly polar compound(s). The MS of the crystalline material indicated a single compound with an apparent M^+ at *m/e* 174, having the composition $C_{11}H_{14}N_2$. Its fragmentation pattern was unlike that of any quinolizidine alkaloid but was characteristic of a substituted indole.[20] This compound was identical with gramine (7), which, though not a typical lupin alkaloid, has been reported in *L. luteus*[21] and *L. hispanicus*.[22,23] Seeds of *L. hartwegii* contained only traces of gramine which increased steadily during growth so that by flowering, it exceeded the amount of lupanine. Finally gramine proved to be different from the previously reported "unknown A".[7]

The presence of aphylline was somewhat surprising since it has never been reported in any legume but rather from *Anabasis aphylla* (family *Chenopodiaceae*).[24] On the other hand, neither epiaphylline nor 10,17-dioxosparteine have been reported in any plant. The apparent absence of the tricyclic base angustifoline is interesting as it is thought to be an oxidation product of 13-hydroxylupanine[4] and their co-occurrence has been commonplace.[25] The taxonomic significance of this unusual alkaloid spectrum would be difficult to assess at present since *L. hartwegii* is not a wild species but a highly crossbred cultivar. Also, since with the exception of our recent studies on fresh plant material[13] most alkaloid reports are from dried flowering plants, many species on reexamination may yield a more surprising alkaloid spectrum.

Since sparteine, α-isosparteine, and β-isosparteine (8) are key compounds in the establishment of the basic ring skeleton and stereochemistry of many alkaloids in this family, it is of interest that all three may be easily separated by GLC or TLC (system B) (Table I) and may be distinguished by their mass spectra.[10,12] Although to date, with the exception of β-isosparteine and lupanoline,[26] no alkaloids with the trans,trans configuration have been isolated from plants, without this information, it could not have been ruled out in the present investigation, given the small amounts of alkaloid or their reduction products.

Finally the GLC behavior of the isomeric pairs sparteine/α-isosparteine and aphylline/epiaphylline was consistent with that observed for lupanine/α-isolupanine and reported for anagyrine/thermopsine,[15] the cis,cis isomer always emerging prior to that with the cis,trans configuration. This observation, together with the clear resolution of the three sparteine isomers, would seem to justify a prediction that all three possible isomers of any alkaloid of this basic skeleton would be resolvable by GLC under similar conditions.

Experimental Section

All solvents were reagent grade, redistilled through a 70-cm column filled with glass helices.

Chromatographic Methods. Three systems previously described[15] and designated as A, B, and C (silica gel G, basic alumina, and cellulose, respectively) were used. Alkaloid zones were located by spraying with iodoplatinate reagent.[27] GLC was carried out using the apparatus, column, and conditions described earlier[13] except that 2-ft glass columns were used and the oven was programmed immediately after sample injection at 2 °C/min. Collection of individual effluent peaks for spectroscopic examination, rechromatography, etc., was achieved as described earlier.[13,15]

Reference Compounds. With the exceptions listed below, all authentic reference alkaloids, their purity, identity, and behavior on all the above chromatographic systems have been described previously.[13,15] An authentic sample of virgiline (13-hydroxyaphylline) and *O*-(2-pyrrolylcarbonyl)virgiline were kindly supplied by Dr. E. P. White of the New Zealand Department of Agriculture, Hamilton, New Zealand. Both were purified by TLC, then by GLC. α-Isosparteine was synthesized in 90% yield by catalytic hydrogenation of thermopsine[28] and purified by preparative TLC (system A), then by GLC. β-Isosparteine was a gift from Dr. M. Carmack, Department of Chemistry, University of Indiana, Bloomington, Ind.

The three isomers of sparteine, crucial to establishing the basic ring structure and stereochemistry of several alkaloids, were separable by GLC or TLC (Table I). Their, MS, published in detail elsewhere,[12,29] while giving similar fragmentation patterns, showed significantly different intensities of the major ions. Sparteine: *m/e* 137 (100), 234 (81), 98 (78), 193 (40), 136 (31), 97 (30). α-Isosparteine: *m/e* 98 (100), 137 (54), 234 (52), 136 (28), 97 (26), 193 (18). β-Isosparteine: *m/e* 137 (100), 98 (99), 234 (70), 97 (62), 136 (46), 193 (31).

Spectroscopic Methods. All integer MS were recorded at 70 eV, using an AEI MS 12 instrument. Samples contained in glass capillary tubes were directly inserted by means of a probe into the ion source. High-resolution mass spectra were carried out by Professor A. M. Hogg, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada. The uv absorbtion spectra were determined in 95% ethanol using a Cary Model 15 instrument. The NMR spectra were determined in $CDCl_3$, with tetramethylsilane as an internal reference, using a Varian HA-100 instrument.

Plant Material. Seeds of *L. hartwegii* were purchased from Herbst Bros., Seedsmen, Brewster, N.Y. The identity of plants grown to flowering was kindly checked by Dr. A. Skoglund of the Crop Science Department, and a voucher specimen, grown to flowering, is on file at the W. P. Fraser Herbarium at the University of Saskatchewan. All plants were grown in vermiculite in a controlled environment chamber under conditions described earlier.[7]

Isolation and Characterization of Plant Alkaloids. Extraction. Freshly harvested, 6 week old plants (640 g) were extracted immediately. Whole plants washed free of vermiculite were homogenized and extracted for basic compounds as described earlier.[13] The pale yellow alkaloid residue, 905 mg as acetate salts, was dried and stored under nitrogen at 0 °C. Recovery of all the reference alkaloids by this scheme was greater than 90%.[13,15]

Identification and Estimation of Alkaloids. Preparative TLC (system A) revealed the presence of eight to ten components. The TLC plates were divided into six zones, designed A–F, visualized by iodoplatinate reagent, collected with a Desaga zone collector, and eluted with 0.5 N HCl and enough sodium sulfite to dispel any purple color. Each of the six fractions was reextracted for basic compounds, then in turn analyzed by GLC, the alkaloids collected,[13] and designated by zone and order of elution from GLC (Table I). The purity of each GLC peak was checked by rechromatography on GLC, all three TLC systems, and by MS.

A1 (Epiaphylline), A2 (Aphylline), and A3. The alkaloids recovered from zone A, then collected from GLC, yielded 30 mg of A1 (white crystals), 20 mg of A2 (colorless liquid), and 1 mg of A3 (pale yellow liquid, rapidly turned red-brown in air). A3, appearing as a shoulder following A2, was freed of any residual A2 by rechromatography on GLC at 190 °C isothermal.

A1: MS M^+ *m/e* 248 (70), 247 (46), 220 (45), 137 (47), 136 (100), 98 (44), 97 (53), 96 (45), published in detail;[28] high-resolution MS M^+ 248.1884 (calcd for $C_{14}H_{24}N_2O$, 248.1889), M − 28 220.1938 (calcd for $C_{14}H_{24}N_2$, 220.1940). A1 (5 mg) was recovered unchanged after hydrogenation over PtO_2 in 1 N HCl at STP for 12 h. Rehydrogenation at 80 °C, 500 psig, yielded 4 mg of a single product identical with α-isosparteine by TLC (system B), GLC, and MS. 17-Oxosparteine was unchanged under either of these hydrogenation conditions.

A2: MS M^+ *m/e* 248 (80), 247 (45), 220 (33), 137 (49), 136 (100), 98 (34), 97 (47), 96 (37), published in detail;[29] high-resolution. MS M^+ 248.1884 (calcd for $C_{15}H_{24}N_2O$, 248.1889), M − 28 220.1938 (calcd for $C_{14}H_{24}N_2$, 220.1940). Hydrogenation of 5 mg of A2 over PtO_2 in 1 N HCl at STP for 12 h gave 4 mg of a single product identical with sparteine by TLC (system B), GLC, and MS.

A3: MS M^+ *m/e* 246 (100), 245 (27), 149 (19), 134 (26), 86 (37), 55 (18). See Table I for TLC and GLC behavior. Insufficient sample was present to characterize further.

B1 (α-Isolupanine), B2, and B3 (10,17-Dioxosparteine). Approximately 6.5 mg of B1 (white crystals), 2.5 mg of B2 (colorless liquid), and 1.5 mg of B3 (colorless liquid) were collected from GLC

of zone B. B1 and B2 were separated from one another on GLC at 200 °C, isothermal. B1 was homogeneous and indistiguishable from an authentic sample of α-isolupanine by TLC, GLC, and MS.[12] B2: MS M+ *m/e* 264 (3), 228 (11), 224 (15), 223 (100), 108 (26), 96 (11), 58 (51), metastables 188.4, 73.5, and 52.3. The sample was not further characterized. B3: MS M+ *m/e* 262 (88), 152 (52), 150 (100), 84 (70), 55 (29), published in detail.[29] Catalytic reduction of 0.5 mg of B3 in 1 N HCl, 500 psig at 10 °C for 24 h, yielded a single product identical by TLC, GLC, and MS with an authentic sample of 17-oxosparteine.

C (Lupanine). A substance (272 mg) was collected from zone C which was homogeneous and identical on all chromatographic systems and by MS[12] with an authentic sample of lupanine. This compound decreased from 85% of the total alkaloid in the seeds to about 27% at flowering.

D1 (Nuttalline), D2 (Virgiline), D3, D4, and D5. D1 (45 mg), D2 (5.5 mg), D3 (0.5 mg), D4 (1.0 mg), and D5 (25 mg), all colorless liquids, were collected by GLC of the alkaloids eluted from zone D. Angustifoline, if present, should have been found in this zone[15] but none was detected. D1 was homogeneous and indistinguishable by TLC, GLC, or MS[10] from an authentic sample of nuttalline (4-hydroxylupanine). D2 was homogeneous on all TLC systems and by GLC. MS M+ *m/e* 264 (35), 246 (24), 236 (34), 154 (25), 153 (74), 152 (100), 123 (26), 84 (51), 55 (31), was indistinguishable from that of an authentic sample of virgiline (10-oxo,13-hydroxysparteine/13-hydroxyaphylline).[29]

D3 and D4 were present in amounts only sufficient to determine their GLC and TLC behavior. D5 was homogeneous on all TLC systems and GLC: MS M+ *m/e* 378 (6), 361 (3), 279 (44), 278 (100), 261 (16), 148 (10), 134 (20), 55 (21). A very large metastable appeared at *m/e* 204.5 with others at *m/e* 343, 245, and 64.7. Alkaline hydrolysis of 4 mg of D5 in 1 N methanolic NaOH at 100 °C for 2 h yielded no recoverable basic material. No basic material was recoverable even after hydrolysis for only 20 min at 50 °C.

E (13-Hydroxylupanine). A substance (1 mg) collected from zone E was homogenous and identical on all TLC systems, GLC, and by MS[12] with an authentic sample of 13-hydroxylupanine. Lupinine, if present, should have been found in this zone[15] but none was detected.

F (Gramine). Sparteine, if present, should have been detected in zone F, but was not. However, 90 mg of a substance was collected which was homogenous on all TLC systems but showed signs of severe decomposition on GLC. Recrystallization from hot acetone gave 85 mg of a white material, mp 128–129 °C. Authentic gramine, similarly recrystallized, had mp 132 °C (lit.[30] 134 °C). MS M+ *m/e* 174 (23), 131 (19), 130 (100), 77 (13), 45 (10), 44 (23) was identical with that of gramine,[29] with metastables at *m/e* 126, 98.6, 81.7, and 57.6; λ_{max} (EtOH) 218 nm (log ε 3.4); ν_{max} 3480 cm^{-1} (s, NH stretch); NMR δ 2.30 s (6 H), 3.70 s (4 H), 7.2 m (4 H); high-resolution MS *m/e* 174.1150 (calcd for $C_{11}H_{14}N_2$; 174.1157), 131.0732 (calcd for C_9H_9N, 131.0735), 103.0540 (calcd for C_8H_5N, 103.0547), 77.0392 (calcd for C_6H_5, 77.0391).

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Registry No.—α-Isoparteine, 446-95-7; sparteine, 90-39-1; β-isosparteine, 24915-04-6; gramine, 87-52-5; 17-oxosparteine, 489-72-5; epiaphylline, 1218-51-5; α-isolupanine, 486-87-3; lupanine, 550-90-3; aphylline, 10159-81-6; nuttalline, 23360-87-4; 13-hydroxylupanine, 15358-48-2; 10,17-dioxosparteine, 52717-73-4; virgiline, 2636-61-5; 17-oxolupanine, 4697-83-0; *O*-(2-pyrrolylcarbonyl)virgiline, 18526-91-5.

References and Notes

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