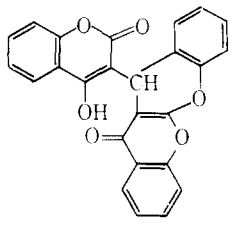
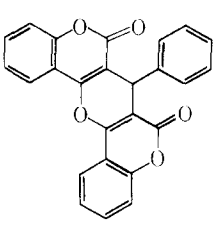
TABLE III
ANTICOAGULANT ACTIVITY OF DICOUMAROLS

No.	Derivative of 4-hydroxycoumarin	Onset, hr	Action peak, hr	Duration, hr	Coagulation valency, %
1	Dicoumarol (ref compd)	24	120	216	10
2	3,3′-Benzylidenebis-	24	48	72	20
3	3,3′-(*o*-Chlorobenzylidene)bis-	24	48	96	35
4	3,3′-(*p*-Chlorobenzylidene)bis-	24	48	120	10
5	3,3′-(*o*-Nitrobenzylidene)bis-	24	48	144	20
6	3,3′-(*p*-Nitrobenzylidene)bis-	24	24	72	20
7	3,3′-(*m*-Hydroxybenzylidene)bis-		Inactive		100
8	3,3′-(*p*-Hydroxybenzylidene)bis-	24	48	72	25
9	3,3′-(*o*-Methoxybenzylidene)bis-	24	48	72	20
10	3,3′-(*m*-Methoxybenzylidene)bis-	24	48	120	10
11	3,3′-(*p*-Methoxybenzylidene)bis-	24	48	96	5
12	3,3′-(*p*-Dimethylaminobenzylidene)bis-	24	48	72	35
13	3,3′-Cinnamylidenebis-	24	48	144	10
14	3,3′-(3-Hydroxy-4-methoxybenzylidene)bis-	24	48	144	15
15	3,3′-(4-Hydroxy-3-methoxybenzylidene)bis-	24	48	72	20
16	3,3′-(2,4-Dimethoxybenzylidene)bis-	24	72	86	20
17	3,3′-(3,4-Methylenedioxybenzylidene)bis-	24	48	72	15
18	3-[6-Oxo-6*H*-benzopyrano[4,3-*b*]-(1)-benzopyran-7-yl]-	24	48	144	5
19	3-[6-Oxo-6*H*-benzopyrano[4,3-*b*]-(1)-11-methoxybenzopyran-7-yl]-	24	48	96	35
20	Anhydride of 3,3′-(*p*-methoxybenzylidene)bis-		Inactive		100
21	4-Hydroxycoumarinyl-2,3,5,6-(3′,4′,3″,4″-dicoumarinopyran-2,5-diene)		Inactive		100



XI



XII

The product of formylation of 4-hydroxycoumarin mentioned earlier is an anhydride and is analogous to the compounds (*e.g.*, XII) prepared by subjecting the various substituted dicoumarols to dehydration. These are inactive.

A significant feature of X and XII is their similarity in shape to the hypothetical ketal from dicoumarol VI. However, there is a difference, namely, the absence of the ketal group in the former, and this seems to lead to inactivity. If, however, the active form of dicoumarol is the lactol VII, the inactivity of the anhydrides can be explained merely by gross structural differences. Further studies are necessary to define the exact structure of the active form.

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Leguminosae Alkaloids. II. Alkaloids of *Lupinus westianus* Small[1]

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As part of our continuing program of examination of alkaloids elaborated by previously uninvestigated or partially investigated plant species of genera belonging to the family *Leguminosae*, we now report the results of our study of the plant, *Lupinus westianus* Small.[3] The only mention of this plant in the chemical literature is that due to Wall and co-workers,[4] who had included it in a large plant survey. Apart from noting the presence of alkaloids in *L. westianus*, however, Wall, *et al.*,[4] did not carry out any chemical investigations.

The plant material used in the present study was collected during the spring of 1962 near Panama City, Fla.[5] The extraction procedure and the methods used for separation and identification of the alkaloids are detailed in the Experimental Section. As it turned out, the three alkaloids elaborated by *L. westianus*, (−)-sparteine, (−)-lupinine, and (−)-multiflorine, constituting 0.16, 0.09, and 0.52% of the weight of the moisture-free plant, respectively, are all known substances. However, a mixture containing the three components, sparteine, lupinine, and multiflorine in 21, 16, and 63 mole %, respectively, was examined for physiological activity in two specific tests.

In the spontaneous activity test, the mixture was injected (intraperitoneal) into Swiss-Webster mice at dosage levels of 1, 5, and 50 mg/kg. Each dosage level was administered to four groups of 5 mice. The mice were placed in a photocell activity cage 1 hr after drug administration, and a 15-min test interval was measured. An identical procedure was followed with a control (saline solution) group of mice. The ratio

(1) For Part I see, S. I. Goldberg and R. F. Moares, *Phytochemistry*, in press.

(2) Taken in part from the dissertation submitted by M. S. S. in partial fulfillment of the Graduate School requirements for the Ph.D. in chemistry, University of South Carolina.

(3) Some taxonomists, as does G. H. M. Lawrence ("Taxonomy of Vascular Plants," The Macmillan Co., New York, N. Y., 1951, p 545 ff), classify the genus *Lupinus* under the subfamily *Lotoideae* or *Papilionaceae*.

(4) M. E. Wall, J. W. Garvin, J. J. Willaman, Q. Jones, B. G. Schubert, and R. A. Davidson, *J. Pharm. Sci.*, **50**, 1001 (1961).

(5) We are indebted to Professor Robert Godfrey of the Florida State University for his expert identification of *L. westianus* and for his aid in arranging for collection and shipments of the plants.

of average control group activity to the average activity of each drugged group was found to be 0.69, 0.40, and 0.41. These results showed the varied effects characteristic of minor depressants which produce ataxia. In the hexobarbital sleeping time test, the alkaloid mixture was administered (intraperitoneal) in doses of 5 mg/kg to groups of 10 mice. One hour later, hexobarbital (100 mg/kg) was administered, and the resulting sleeping time was measured and compared to a saline control group. The results showed a potentiation of hexobarbital sleeping time in that the average sleeping time of the drugged mice was found to be 50 min while that of the control group was 13 min. Both of these preliminary tests gave, therefore, indication of CNS activity and may be interpreted to mean that the mixture of alkaloids obtained from *L. westianus* contains one or more depressant constituents of a weak nature.

In addition, pure, crystalline lupinine was evaluated for its action on isolated uterine strips. The results of this assay showed lupinine to possess oxytocic activity only to a minor degree. A pure sample of (−)-17-oxosparteine was also tested for its oxytocic activity and found to exhibit only about 20% of the oxytocic activity of sparteine sulfate.

Experimental Section[6]

Alkaloids of *Lupinus westianus* Nutt.—The whole plants (including roots) which were collected while in full bloom during April 1962, near Panama City, Fla., were air-dried for 2 weeks and then milled to about 80 mesh. This material was used for all of our extraction work. It was determined to possess an average moisture content of 7%. Three kilograms was moistened with 6 *N* NH_4OH solution and loosely packed into a sack made of bleached muslin. The sack and contents were immersed in chloroform, and the extraction was allowed to proceed during 24 hr with occasional stirring of the $CHCl_3$ infusion. In this manner three 1-day batch extractions were carried out. After the three extracts were combined and condensed to approximately 4 l., the dark green residue was successively washed with 6 *N* HCl. The combined acidic washes were made strongly basic with 6 *N* NaOH and then extracted ($CHCl_3$). The chloroform extracts were combined, dried (Na_2SO_4), filtered, and evaporated *in vacuo* to provide the mixture of crude alkaloids as a red-brown colored, viscous oil. A total of 80 g of crude alkaloids was accumulated in this way by the batchwise extraction of 9.7 kg of *L. westianus*. Paper chromatographic examination of the crude alkaloid mixture gave three spots: R_f 0.53, 0.42, and 0.22, shown later to be sparteine, lupinine, and multiflorine, respectively.

Fractional distillation of the mixture of crude alkaloids provided sparteine and lupinine. Multiflorine was obtained by column chromatography of the distillation residue on Woelm, nonalkaline, alumina. The lower boiling fraction [bp 94° (0.9 mm)] exhibited an infrared spectrum (neat) which was found to be superimposable upon that obtained from authentic sparteine.[8] Both substances also displayed identical chromatographic behavior, and the specific rotation ($[\alpha]^{27}D$ −16.8 ± 0.2° (*c* 6.03, ethanol)) determined from the plant alkaloid closely matched that ($[\alpha]D$ −17.0° (ethanol)) previously reported[9] for sparteine. In addition, a monoperchlorate salt (mp 169.0–169.5°) and a monohydriodide salt (mp 229–231°) were prepared from the plant alkaloid; mixture melting point with the corresponding salts prepared from authentic sparteine,[8] showed no depression. The higher boiling fraction [bp 113° (0.8 mm)] obtained from distillation of the mixture of crude plant alkaloids slowly crystallized after several hours. This material was recrystallized from hexane several times to provide a constant melting product. The product was shown to be **lupinine** by means of elemental analysis and comparison of its properties (mp 68.0–68.5°, $[\alpha]^{23}D$ −23.2 ± 0.3° (*c* 3.77, ethanol), hydrochloride mp 213.5–214.0°) with those reported[9] for lupinine (mp 70–71°, $[\alpha]D$ −21.3° (ethanol), hydrochloride mp 207–209°). In addition, an infrared spectrum (CCl_4) determined from the plant alkaloid was found to be superimposable upon that obtained from a sample of authentic lupinine.[10]

Anal. Calcd for $C_{10}H_{19}NO$: C, 70.96; H, 11.31; N, 8.28. Found: C, 70.62; H, 11.67; N, 8.57.

The individual base (R_f 0.22) obtained from the ethyl acetate eluates of alumina chromatography of the distillation residue was shown to be multiflorine. The material, initially obtained as a viscous, slightly yellow oil, was molecularly distilled [160–196° (air bath, 0.06 mm)] to yield a colorless oil which was induced to crystallize from hexane. The product was recrystallized until constant mp 107–108°. This melting point, along with its specific rotation ($[\alpha]^{26}D$ −310 ± 1.0° (*c* 5.03, methanol)) and spectral properties [infrared, $\nu_{max}^{CHCl_3}$ 1640 (conjugated amide carbonyl)[11] and 1590 cm^{-1} (conjugated olefin);[11] ultraviolet, $\lambda_{max}^{ethanol}$ 248 and 325 mμ (ε 7800 and 12,400); nmr, δ^{CDCl_3} 6.89 (1 H doublet, J = 13 cps) and 4.94 (1 H doublet, J = 13 cps)] were strongly reminiscent of properties displayed by multiflorine, an alkaloid recently found in this laboratory[1] to be among those elaborated by *L. diffusus*. The identity with multiflorine was readily confirmed by means of mixture melting point and superimposable infrared spectra.

The weight percentage of each alkaloid was determined by careful extraction of 30.0 g of dry, milled whole plant. The crude mixture of alkaloids obtained amounted to 0.316 g, or 1.14% of the weight of the moisture-free plant. A sample (0.267 g) of this material was passed through a column of Woelm, nonalkaline alumina (Brockmann grade I) in ethyl acetate to remove the highly polar colored impurities. The column was washed with ethyl acetate until no more basic material was obtained in the eluent. Evaporation of the ethyl acetate left 0.180 g of the slightly yellow alkaloid mixture. Based upon this determination, the alkaloid content of *L. westianus* was calculated as 0.77% of the weight of the whole, moisture-free plant. The relative amounts of each alkaloid were then determined by measurement of the peak areas obtained from gas–liquid partition chromatographic analysis[12] of the alkaloid mixture. It was previously established, using a known mixture of the three alkaloids, that the ratio of integrated peak areas accurately reflected the weight ratio of the alkaloids. In this manner it was determined that sparteine, lupinine, and multiflorine occurred in *L. westianus* to the extent of 0.16, 0.09, and 0.52%, respectively, of the weight of the moisture-free whole plant.

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(6) Temperature readings are uncorrected. Melting points were determined with samples in evacuated sealed capillary tubes. Combustion analyses are by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Whatman No. 1 paper with a solvent system of 1-butanol–acetic acid (10:1, v/v) that had been saturated with water was used for the descending paper chromatography reported herein. The papergrams were allowed to develop until the solvent front had gone approximately 24 cm from the starting line. Spots were visualized by application (spraying) of Dragendorff reagent.[7]

(7) "The Merck Index," 5th ed, Merck and Co., Inc., Rahway, N. J., 1940, p 696.

(8) Prepared from commercially available (Inland Alkaloid Co.) sparteine sulfate.

(9) N. J. Leonard, *Alkaloids*, **3**, 126 (1953).

(10) We are indebted to Dr. Bryce Douglas, Smith Kline and French Laboratories, Philadelphia, Pa., for this sample.

(11) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1958; K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, Inc., San Francisco, Calif., 1962.

(12) Carried out on a F & M Scientific Corp., Model 500, gas chromatograph with an 8 ft × 0.25 in. cooper column packed with Diatoport-W containing 5% (w/w) General Electric, SE-30 silicone rubber. Helium, 3.5 kg/cm², was used as carrier gas, and the column was temperature programmed between 150 and 200° during the analyses.