THE ALKALOIDS OF *DELPHINIUM CASHMIRIANUM*

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ABSTRACT.—*Dephinium cashmirianum* Royle (Ranunculaceae) has yielded the new base cashmiradelphine (**12**), together with the known alkaloids anthranoyllycoctonine (**9**), lycaconitine (**15**), avadharidine (**17**), lappaconitine (**4**), and *N*-deacetyllappaconitine (**7**). Pyridinium chlorochromate oxidation of lycoctonine furnished the new aldehyde lycoctonal (**11**). The arrhythmogenic and heart rate effects of several of these diterpenoidal alkaloids have been measured on the isolated guinea atria. Lappaconitine was arrhythmogenic at 10^{-4}M concentrations. But in contrast to the reference drug aconitine, lappaconitine did not increase the heart rate. In anesthetized rabbits injected with lappaconitine, *N*-deacetyllappaconitine, and lappaconine up to 1 mg/kg, cardiac arrhythmia was quickly observed. Even up to 5 mg/kg, the other substances were non-arrhythmogenic.

A recent investigation of the alkaloids of the dwarf larkspur, *Delphinium tricorne* Michaux (Ranunculaceae), has yielded the well known alkaloid methyllycaconitine (**1**), as well as an inseparable mixture of alkaloidal artifacts consisting of structures **2** and **3** formed from **1** during treatment with ammonium hydroxide (1). Additionally, the alkaloid "delsemine," obtained from the Russian species *D. semibarbatum* and originally assigned alternative structures **2** or **3**, (2, 3), was shown also to be an artifact of isolation and to consist of a mixture of compounds **2** and **3**.

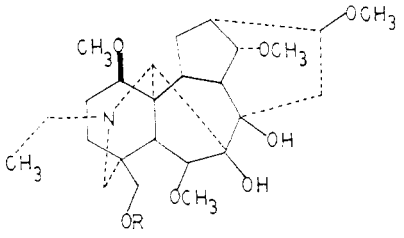
In the present study, the ethanol extracts from *D. cashmirianum* Royle collected in Kashmir, Pakistan, were chromatographed on a silica gel column with chloroform and chloroform-methanol mixtures as eluent. Elution with chloroform supplied the known (+)-lappaconitine (**4**) (4) the basic hydrolysis of which yielded lappaconine (**5**) and *N*-acetylanthranilic acid (**6**) (5, 6, 7).

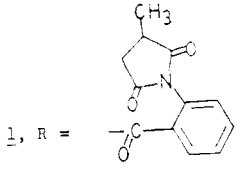
Further elution of the column with 2% methanol in chloroform provided additional quantities of (+)-lappaconitine (**4**) together with the known (+)-*N*-deacetyllappaconitine (**7**) (8). Hydrolysis of the latter alkaloid led to lappaconine (**5**) and anthranilic acid (**8**). It should be noted that this is the first report of the isolation of **4** and **7** from a *Delphinium* species; these had been found previously only in *Aconitum* species.

Three compounds were found in the 5% methanol in chloroform eluate. The first was (+)-anthranoyllycoctonine (**9**) the hydrolysis of which afforded (+)-lycoctonine (**10**) and anthranilic acid (**8**). When **9** and **10** were independently subjected to pyridium chlorochromate in methylene chloride treatment, **10** but not **9** underwent oxidation to the new aldehyde **11**, (+)-lycoctonal, confirming that the masked primary alcohol function of **10** is esterified.

The second compound obtained from the 5% methanol in chloroform eluate was the new compound (+)-cashmiradelphine (**12**), the ir spectrum of which showed amide (1680 cm^{-1}), aromatic ester (1710 cm^{-1}), and aliphatic ester (1740 cm^{-1})

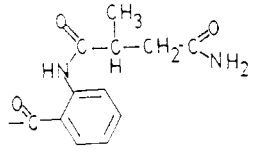
peaks. Hydrolysis of **12** gave rise to (+)-lycoctonine (**10**) and the acid **13**, which corresponds to the hitherto unknown *o*-(*N*-succinimidyl)benzoic acid. Lithium aluminum hydride reduction of (+)-cashmiradelphine (**12**) led to (+)-lycoctonine (**10**) and the amino alcohol **14**. Pyridinium chlorochromate oxidation of cashmiradelphine did not yield any well-defined product, pointing to the fact that no free primary alcohol is present in the molecule. Finally, condensation of anthranoyllycoctonine (**9**) with the acid chloride of monomethyl succinate furnished material identical in all respects with cashmiradelphine (**12**).



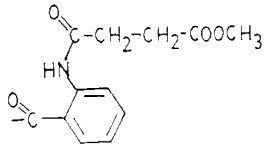


1, R =

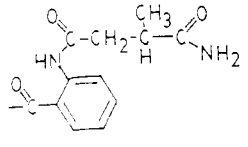
10, R = H



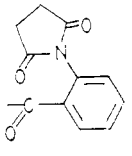
2, R =



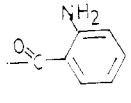
12, R =



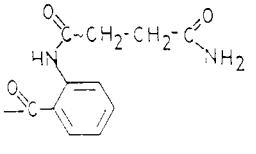
3, R =



15, R =



9, R =



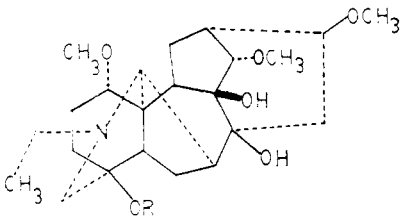
17, R =

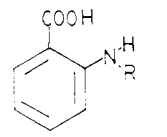
The third alkaloid from the 5% methanol in the chloroform fraction was determined to be (+)-lycaconitine (**15**) (3). Hydrolysis of **15** afforded lycoctonine (**10**) and the acid **13**, whereas lithium aluminum hydride reduction of **15** provided **10** and the amino alcohol **16**.

The column eluate consisting of 10% methanol in chloroform furnished (+)-avadharidine (**17**) as a colorless, amorphous, powder. Its lithium aluminum hydride reduction gave rise to lycoctonine (**10**).

It has been previously pointed out that avadharidine (**17**) could be an artifact of isolation, derived by aminolysis of lycaconitine (**15**) (3). In view of this observation, and of the isolation of artifacts **2** and **3** from *D. tricorne* and *D. semibarbatum*, one is justified in questioning whether cashmiradelphine (**12**) is a true

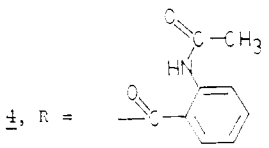
alkaloid. This compound, however, is probably a natural product because the original plant extraction was carried out with ethanol, and the crude plant extracts revealed the presence of cashmiradelphine (**12**) by tlc prior to column chromatography. On the other hand, it should also be stated that the imide ring in





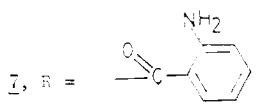
6, R = $-C(=O)-CH_3$

5, R = H

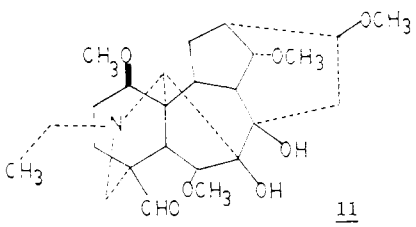


4, R =

5, R = H



7, R =



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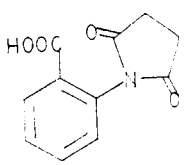

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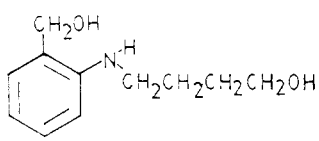

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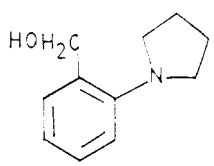

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lycaconitine (**15**) can be very readily cleaved, as indicated by our finding that, upon standing over silica gel or alumina in methanol for 16 hr, lycaconitine (**15**) solvolyzed to produce cashmiradelphine (**12**). Alternatively, refluxing lycaconitine in methanol for one hour also generated cashmiradelphine.

CHEMICAL STUDY

EXPERIMENTAL[1]

EXTRACTION AND PRELIMINARY FRACTIONATION.—Ground roots of *Delphinium cashmirianum* Royle[2] (2.5 kg) were extracted with ethanol. When the solvent was evaporated, a thick residue (150 g) resulted.

[1]Melting points are uncorrected. All tlc were on Merck 254 silica gel plates. Pmr spectra are at 60 MHz.

[2]The *Delphinium cahsmirianum* plant was collected by Mr. Jan Mohammod, research assistant at Gomal University, in the Neelam Valley of Azad Kashmir, Pakistan; and was identified by Dr. A. R. Beg, Plant Taxonomist, Forest Research Institute, University of Peshawar, Peshawar, Pakistan.

The crude ethanolic extract (90 g) was loaded on a silica gel (Merck 70–230 mesh) column, with chloroform as the first solvent. As the column was continuously eluted with increasingly polar mixtures of methanol and chloroform, the following fractions were collected: Fraction I: chloroform; Fraction II: 2% methanol-chloroform; Fraction III: 5% methanol-chloroform; Fraction IV: 10% methanol-chloroform.

(+)-LAPPACONITINE (**4**).—Fraction I, upon evaporation and recrystallization, furnished (+)-lappaconitine (**4**) (10 g), mp 219–221° (ether) (lit. 227° from ether) (10); $[\alpha]^{27}D+20°$ (c 0.5 EtOH); ir ($CHCl_3$) 1685, 1695, 3250, and 3500 cm^{-1}; pmr ($CDCl_3$) δ 1.11 (3H, t, $J=7$ Hz, CH_3CH_2N), 2.18 (3H, s, CH_3CO), 3.27 (6H, s, OCH_3), 3.38 (3H, s, OCH_3), 6.93 (1H, t, $J=8$ Hz, ArH), 7.35 (1H, t, $J=8$ Hz, ArH), 7.85 (1H, d, $J=8$ Hz, ArH), 8.58 (1H, d, $J=8$ Hz, ArH), and 10.90 (1H, br s, NH).

Anal. high res. ms, calc. for $C_{32}H_{44}N_2O_8$: 584.3095. Fd. 584.3073.

The above data compare favorably with those reported for (+)-lappaconitine (9).

HYDROLYSIS OF **4**.—Alkaloid **4** (15 mg, 0.03 mmol) was refluxed with ten ml of methanolic K_2CO_3 for four hr. Work-up gave (+)-lappaconine (**5**) (9 mg, 83%); ir ($CHCl_3$) 3400–3500 and 3600 cm^{-1}; and *N*-acetylanthranilic acid (**6**), mp 185° (MeOH); ir (KBr) 1650, 1690, 2500, 2940, 3140, and 3375 cm^{-1}; pmr (TFA) δ 2.43 (3H, s, CH_3CONH), 7.21 (1H, t, $J=8$ Hz, ArH), 7.58 (1H, t, $J=8$ Hz, ArH), 8.08 (1H, d, $J=8$ Hz, ArH), and 8.23 (1H, d, $J=8$ Hz, ArH); ms *m/e* 179 (65) (M^-), 138 (90), 119 (base), and 92 (40).

(+)-*N*-DEACETYLLAPPACONITINE (**7**).—Evaporation of fraction II left (+)-**4**, and (+)-*N*-deacetyllappaconitine (**7**) (2 g) purified by tlc with 2% methanol in chloroform; mp 120–121° (MeOH); $[\alpha]^{27}D+42°$ (c 0.34 EtOH); ir ($CHCl_3$) 1662 and 3460 cm^{-1}; pmr ($CDCl_2$) δ 1.13 (3H, t, $J=6.5$ Hz, CH_3CH_2N), 3.28 (3H, s, OCH_3), 3.32 (3H, s, OCH_3), 3.42 (3H, s, OCH_3), 6.49–6.70 (2H, m, ArH), 7.27 (1H, t, $J=8$ Hz, ArH), and 7.81 (1H, d, $J=8$ Hz, ArH).

Anal. high res. ms, calc. for $C_{30}H_{42}N_2O_7$: 542.8919. Fd. 542.2997.

HYDROLYSIS OF **7**.—Hydrolysis of **7** (20 mg, 0.04 mmol) in methanolic K_2CO_3 provided (+)-lappaconine (**5**) (13 mg, 83%) and anthranilic acid (**8**) (4 mg, 80%); mp 143° (MeOH).

(+)-ANTHRANOYLLYCOCTONINE (**9**).—Fraction III was evaporated and was found by tlc to contain three major components which were separated by use of 5% methanol in chloroform. The band (R_f 0.27) with a blue-white fluorescence under uv light corresponded to (+)-anthranoyllycoctonine (**9**) (2 g); mp 132–135° (MeOH), [lit. 132–135 (MeOH) (10)]; $[\alpha]^{27}D+42°$ (c 0.54 EtOH); ir ($CHCl_3$) 1690 and 3500 cm^{-1}; pmr ($CDCl_3$) δ 1.05 (3H, t, $J=7$ Hz, CH_3CH_2N), 3.23 (3H, s, OCH_3), 3.32 (3H, s, OCH_3), 3.35 (3H, s, OCH_3), 3.38 (3H, s, OCH_3), 6.58 (1H, t, $J=7$ Hz, ArH), 7.10 (1H, t, $J=7$ Hz, ArH), 7.75 (1H, d, $J=$Hz, ArH), and 8.27 (1H, d, $J=7$ Hz, ArH).

Anal. high res. ms, calc. for $C_{32}H_{44}N_2O_7$: 568.3147. Fd. 568.3157.

The above data compare favorably with those reported for (+)-anthranoyllycoctonine (10).

Compound **9** (20 mg, 0.03 mmol), when hydrolyzed by use of methanolic K_2CO_3, yielded (+)-lycoctonine (**10**) (15 mg, 94%); mp 103–108° (MeOH) [lit. 139° (aq. MeOH) (11) or 96–97° (aq. EtOH)] (10); $[\alpha]^{27}D+47°$ (c 0.40 EtOH); ir ($CHCl_3$) 3440 and 3620 cm^{-1}; identical with an authentic sample; and anthranilic acid (**8**), (4 mg, 85%).

(+)-LYCOCTONAL (**11**).—(+)-Lycoctonine (10) (20 mg, 0.04 mmole) in methylene chloride (10 ml) at 0° was treated with pyridinium chlorochromate (32 mg, 0.16 mmol). The mixture was brought to room temperature and stirred for 5 hr. Dilute ammonium hydroxide was added, and the methylene chloride layer was separated and evaporated. The product separated by tlc with ammoniated ether as solvent (R_f 0.14) was (+)-lycoctonal (**11**) (7 mg, 35%); mp 73° (MeOH); $[\alpha]^{27}D+74°$ (c 0.59 EtOH); ir ($CHCl_3$) 1722 and 3440 cm^{-1}; pmr ($CDCl_3$) δ 1.06 (3H, t, $J=7$ Hz, CH_3CH_2N), 3.23 (3H, s, OCH_3), 3.26 (3H, s, OCH_3), 3.30 (3H, s, OCH_3), 3.37 (3H, s, OCH_3), and 9.38 (1H, s, CHO); ms *m/e* 465 (80) (M^+) ($C_{25}H_{39}NO_7$), 450 (80) $(M-CH_3)^+$, 434 (base) $(M-OCH_3)^-$, and 406 (75) $(434-CO)^+$.

Anal. high res. ms, calc. for $C_{25}H_{39}NO_7$: 465.2725. Fd. 465.2733.

(+)-CASHMIRADELPHINE[3] (**12**).—The band with R_f 0.22 from fraction III corresponded to the new base (+)-cashmiradelphine (**12**) (2 g); mp 130–135° (methanol-ether); $[\alpha]^{27}D+56°$ (c 0.39 EtOH); λ max (EtOH) 253 and 310 nm (log ϵ 4.15 and 3.74); ir ($CHCl_3$) 1680, 1710, 1740, 3260 and 3420 cm^{-1}; pmr ($CDCl_3$) δ 1.06 (3H, t, $J=7$ Hz, CH_3CH_2N), 2.72 (4H, s, $CH_2CH_2COOCH_3$), 3.20 (3H, s, OCH_3), 3.28 (3H, s, OCH_3), 3.35 (6H, s, 2 x OCH_3), 3.62 (3H, s, $COOCH_3$), 6.80 (1H, t, $J=8$ Hz, ArH), 7.40 (1H, t, $J=8$ Hz, ArH), 7.83 (1H, d, $J=8$ Hz, ArH), 8.46 (1H, d, $J=8$ Hz, ArH), and 10.75 (1H, br S, HNCO); ms *m/e* 700 (15) (M^+) ($C_{47}H_{52}N_2O_{11}$), 682 (80) $(M-H_2O)^+$, 669 (base) $(M-OCH_3)^+$, 651 (70) $(669-H_2O)^+$, and 450 (50).

Anal. high res. ms, calc. for $C_{37}H_{52}N_2O_{11}$: 700.3569. Fd. 700.3570.

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[3]*Note Added to Accepted Manuscript*: We have discovered that our cashmiradelphine corresponds to septentriodine, recently isolated from *Aconitum septentrionale* Koelle.: S. W. Pelletier, R. S. Sawhney and A. J. Aasen, *Heterocycles*, **12**, 377 (1979).

HYDROLYSIS OF **12**.—(+)-Cashmiradelphine (**12**) (20 mg, 0.02 mmole) was hydrolyzed with methanolic K_2CO_3 to produce (+)-lycoctonine (**10**) (12 mg, 90%) and *o*-(*N*-succinimidyl)benzoic acid (**13**) (5 mg, 80%); mp 170° (MeOH); λ max (EtOH) 252 and 303 nm (log ε 4.24 and 3.72); ir (KBr) 1675 and 3000 cm^{-1}; pmr (TFA) δ 2.98 (4H, s, $COCH_2CH_2CO$), 7.25 (1H, t, J=7 Hz, ArH), 7.65 (1H, t, J=7 Hz, ArH), 8.18 (1H, apparent d, J=7 Hz, ArH), and 8.31 (1H, apparent d, J=7 Hz, ArH); ms *m/e* 219 (42) (M^+), 175 (58) $(M-CO_2)^-$ and 119 (base) $(175-CH_2CH_2CO)^-$.

REDUCTION OF **12**.—(+)-Cashmiradelphine (**12**) (50 mg, 0.07 mmol) was reduced with excess $LiAlH_4$ in hot THF. The products were separated by tlc with ammoniated ether as solvent. The band with R_f 0.25 was identified as (+)-lycoctonine (**10**) (20 mg, 60%). The band with R_f 0.50 proved to be the amino alcohol **14** (8 mg, 57%); λ max (EtOH) 248 and 296 nm (log ε 3.91 and 3.31); ir ($CHCl_3$) 3400 and 3600 cm^{-1}; ms *m/e* 195 (33) (M^-) ($C_{11}H_{17}NO_2$), 136 (30) $(M-CH_2CH_2CH_2OH)^-$, and 118 (base) $(136-H_2O)^-$.

Anal. high res. ms, calc. for $C_{11}H_{17}NO_2$: 195.1258. Fd. 195.1269.

PARTIAL SYNTHESIS OF CASHMIRADELPHINE (**12**).—About 5 mg of (+)-anthranoyllycoctonine (**9**) was treated with monomethylsuccinic acid chloride in ethanol-free chloroform for 16 hr at room temperature. Work-up yielded **12**.

(+)-LYCACONITINE (**15**).—The band with R_f 0.05 from fraction III corresponded to (+)-lycaconitine (**15**) (500 mg); mp 113° (MeOH); $[\alpha]^{27}D$+16° (c 0.64 EtOH); ir ($CHCl_3$) 1705 sh, 1720, and 3450 cm^{-1}; pmr ($CDCl_3$) δ 1.05 (3H, t, J= Hz, CHC_3H_2N), 2.87 (4H, s, $COCH_2CH_2CO$), 3.23 (3H, s, OCH_3), 3.31 (3H, s, OCH_3), 3.33 (3H, s, OCH_3), 3.38 (3H, s, OCH_3), 7.25 (1H, t, J=7 Hz, ArH), 7.58 (1H, t, J=7 Hz, ArH), 8.08 (1H, d, J=7 Hz, ArH), and 8.47 (1H, d, J=7 Hz, ArH).

Anal. high res. ms, calc. for $C_{36}H_{48}N_2O_{10}$: 668.3294. Fd. 668.3186.

The above data compare favorably with those reported for (−)-lycaconitine (3).

HYDROLYSIS OF **15**.—Methanolic K_2CO_3 hydrolysis of **15** (20 mg, 0.03 mmol) yielded (+)-lycoctonine (**10**) (12 mg, 86%) and *o*-(*N*-succinimidyl)benzoic acid (**13**) (5 mg, 76%).

REDUCTION OF **15**.—(+)-Lycaconitine (**15**) (50 mg, 0.07 mmol) was reduced with excess $LiAlH_4$ in hot THF. The products were separated by tlc with ammoniated ether as solvent. The band with R_f 0.25 was collected to obtain (+)-lycoctonine (**10**) (18 mg, 51%). The compound with R_f 0.75 was collected to give the amino alcohol **16** (7 mg, 53%), λ max (EtOH) 253 and 285 sh nm (log ε 3.78 and 3.13); ir ($CHCl_3$) 3400 cm^{-1}.

Anal. high res. ms, calc. for $C_{11}H_{15}NO$: 177.1153. Fd. 177.1154.

(+)-AVADHARIDINE (**17**).—Amorphous (+)-avadharidine (100 mg) was obtained by evaporation of fraction IV, $[\alpha]^{27}D$+40° (c 0.37 EtOH); ir ($CHCl_3$) 1690, 1705, 3300 and 3430 cm^{-1}; pmr ($CDCl_3$) δ 1.05 (3H, t, J=7 Hz, CH_3CH_2N), 3.22 (3H, s, OCH_3), 3.30 (3H, s, OCH_3), 3.33 (3H, s, OCH_3), 3.37 (3H, s, OCH_3), 7.00 (1H, t, J=8 Hz, ArH), 7.46 (1H, t, J=8 Hz, ArH), 7.88 (1H, d, J=8 Hz, ArH), 8.57 (1H, d, J=8 Hz, ArH), and 10.87 (1H, br s, ArN*H*CO); ms *m/e* 668 (8) $(M-NH_3)^+$ ($C_{36}H_{48}N_2O_{10}$) 650 (10) $(668-H_2O)^-$, 637 (40) $(668-OCH_3)^+$, 619 (5) $(637-H_2O)^+$, and 555 (base).

Anal. high res. ms, calc. for $C_{36}H_{46}N_2O_9$ $(M-NH-H_2O)^-$, 650.3199. Fd. 650.3131.

The above data compare favorably with those reported for (+)-avadharidine (3, 12).

HYDROLYSIS OF AVADHARIDINE (**17**).—Methanolic K_2CO_3 hydrolysis of **17** (20 mg, 0.03 mmol) afforded (+)-lycoctonine (**10**) (12 mg, 88%) and the acid **13** (5 mg, 78%).

REDUCTION OF **17**.—Compound **17** (50 mg, 0.07 mmol), when reduced with $LiAlH_4$ in hot THF, yielded (+)-lycoctonine (**10**) (17 mg, 50%).

CARDIOVASCULAR STUDY

METHODS[4]

EXPERIMENTS *in vitro*.

Isolated Guinea-Pig Atria.—Male albino guinea pigs weighing between 350–400 g were used. The animals were killed by a blow on the head and quickly exsanguinated by cutting the neck at the level of the carotid arteries. The heart was isolated and placed in oxygenated Locke solution. The atria were dissected and suspended in oxygenated (95% O_2 and 5% (CO_2) Locke solution in a 10 ml tissue bath which was maintained at 37°C. The composition of the salt solution in milimolar was sodium chloride, 154.0; potassium chloride, 5.63; calcium chloride, 2.10; sodium bicarbonate, 5.96; and dextrose 5.55.

[4]Physiograph 4 for measuring beat of atria and the blood pressure tranducer was from E & M Instruments, Houston, Texas. The anesthetics xylazine was Rompun® and the katamene was Vetalar®. For the solutions all drugs were dissolved in 1 ml of 0.2 N HCl and completed to the required volume with physiological solution (NaCl 8.9%).

The atrium was tied to a glass tissue hook at one end, and the other end of the preparation was connected to the recording system. The spontaneous beat of the atrium was recorded *via* an isometric transducer type A on a Physiograph 4. A resting atrial tension of 1 g was maintained.

EXPERIMENTS *in vivo*.

Albino rabbits, either sex, weighing between 2.5–3.0 kg were used. The animals were obtained from Lane Bailey (Xenia, Ohio). The rabbits were anesthetized with 10 mg/kg of xylazine, I.M., and 5 minutes later 50 mg/kg of ketamine, I.M. In most cases, it was necessary to give a maintenance dose of 10 mg/kg of ketamine 60 minutes after the first dose. The animal was maintained under artificial respiration via a tracheal cannula connected to a respirator. The respiratory rate was maintained at 20 strokes/min. The left carotid artery was isolated and cannulated with a polyethylene tube connected to the recording system. Blood pressure was recorded via a blood pressure transducer.

For the recording of the heart rate, a pair of electrodes was placed at the hind left leg and right fore leg, respectively. The electrodes were connected to a preamplifier, and both the blood pressure and heart rate were recorded on a Physiograph 4. All solutions of drugs were administered through a polyethylene tube connected to a needle placed in the rabbit's ear marginal vein. A larger dose of drug was administered when no effect was observed within five minutes after administration of the previous dose. The appearance of arrhythmias was taken as the end point of the assay. However, in most cases when no response was observed with the administration of a dose of 1 mg/kg of drug, no additional doses were tested.

RESULTS

Experiments in vitro.—Figure 1 shows the heart rate and arrhythmogenic effects of aconitine and four alkaloids obtained from *Delphinium cashmirianum*. Aconitine produced a dose-related increase in heart rate previous to the production of arrhythmias. Arrhythmias were produced at a final bath concentration of 3×10^{-5}M aconitine. Of the *Delphinium* alkaloids tested *in vitro*, only lappaconitine showed an arrhythmogenic effect. The arrhythmias appeared with a dose of 1×10^{-4}M. However, in contrast with aconitine, this effect was not preceded

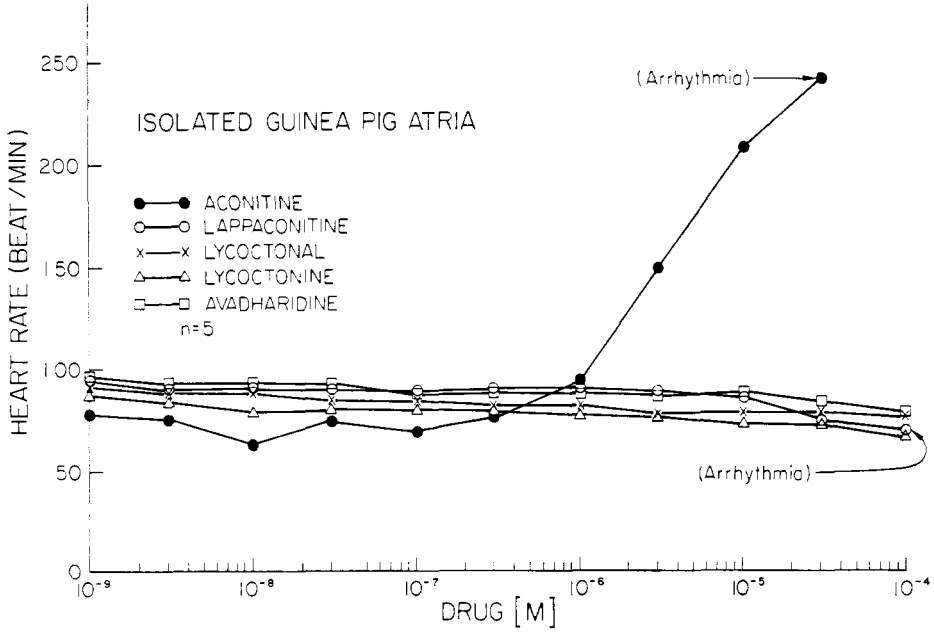

FIG. 1. Effects of the alkaloids from *Dephinium cashmirianum* on the isolated guinea pig atria. At 3×10^{-5}M, aconitine cardiac arrythmia was observed. Although lappaconitine did not change the heart rate, cardiac arrythmia at 10^{-4}M was observed.

TABLE 1. Effects of the alkaloids from *Delphinium cashmirianum* on the rabbit heart rate, *in vivo*. Group I—Arrhythmogenic Substances.

Name	Basal Heart Rate	mg/kg						Dose producing arrhythmias (mg/kg)	Time to produce arrhthmia (sec)
		0.05	0.10	0.25	0.50	1.00	5.00		
		Δ Heart rate beats/min							
Lappaconitine (4)	190	—	—	−10	—	−35	—	1.00	90
	190	—	0	− 5	−30	−40	—	1.00	45
N-Deacetyl-lappaconitine (7)	170	—	−8	+ 5	0	0	—	1.00	52
	180	—	—	—	—	0	—	1.00	85
Lappaconine (5)	185	—	−5	0	0	0	0	—	—

by an increase in heart rate. The arrhythmias produced by lappaconitine disappeared with repeated washings of the tissue and were reestablished with the addition of a dose of 1×10^{-4}M lappaconitine. The aconitine-induced arrhythmias did not disappear with washing of the tissue. The other alkaloids did not produce significant changes in the basal atrial rate.

Experiments in vivo.—Tables 1 and 2 show the effects of *Delphinium cashmirianum* alkaloids on the heart of the rabbit, *in vivo*. Only drugs belonging to group I showed arrhythmogenic activity: namely, lappaconitine and its derivative *N*-deacetyllappaconitine. No changes in the cardiac activity were observed with administration of lappaconine. The arrhythmias induced by lappaconitine

TABLE 2. Effects of the alkaloids from *Delphinium cashmirianum* on the rabbit heart rate, *in vivo*. Group II—Nonarrhythmogenic Substances.

Name	Basal Heart Rate	mg/kg					
		0.05	0.10	0.25	0.50	1.00	5.00
		Δ Heart rate beats/min					
Lycoctonine (**10**)	185	—	−25	—	0	—	—
	200	—	+ 5	+5	0	0	—
Avadharidine (**17**)	180	0	+ 8	+5	0	0	−10
Anthranoyllycoctonine (**9**)	170	—	—	+8	+ 5	− 5	—
Lycaconitine (**15**)	180	—	−10	0	−10	—	—
	195	—	+ 3	+5	0	− 5	—
Cashmiradelphine (**12**)	180	−5	+ 5	−5	− 5	−20	—
	178	—	—	—	− 8	−10	—
Lycoctonal (**11**)	200	−3	0	0	0	—	—
	185	0	− 5	0	0	0	−10

were preceded by a marked decrease in heart rate. This did not happen with *N*-deacetyllappaconitine. The presence of arrhythmias was accompanied by a marked fall in blood pressure. Both effects persisted until the end of the experiment. Lappaconine did not produce any significant change in the blood pressure of the rabbits.

Drugs belonging to group II did not produce any significant changes in heart rate or blood pressure. These drugs did not show any arrhythmogenic effect in the range of doses used. At doses above 0.25 mg/kg, lycaconitine produced a small increase in blood pressure and cashmiradelphine produced a small decrease in blood pressure. These changes, however, were not dose related.

TABLE 3. Effects of aconitine on the rabbit heart rate, *in vivo*.

Name	Basal Heart Rate	Dose mg/kg			Dose producing arrhythmias (mg/kg)	Time to produce arrhythmias (sec)
		0.005	0.010	0.100		
		Δ HR beats/min				
Aconitine.....	180	—	—	+160	0.1	12
	150	+110	+170	—	0.005* 0.010	180 30

*The heart activity was completely normal 30 min after the administration of this dose.

In the range of doses studied, aconitine consistently produced an increase in heart rate previous to the induction of arrhythmias. The arrhythmias induced by 0.005 mg/kg were reversible. At higher doses, the arrhythmias persisted until the end of the experiment. A marked fall in blood pressure was observed together with the arrhythmic activity. At doses above 0.005 mg/kg, the fall in blood pressure was maintained until the end of the experiment.

DISCUSSION

The results presented above show that compounds belonging to group I have arrhythmogenic activity. However, this activity is limited to compounds having an anthranoyl or *N*-substituted anthranoyl groups in position 4 (table 1). As can be seen in table 1, lappaconine does not have a substituent in that position. It also appears that the *N*-acetyl group does not interfere with the arrhythmogenic activity. These drugs, however, are about 100 times less potent than aconitine in the rabbit. Lappaconitine has been reported to be 20–40 times less toxic than aconitine in mice, depending on the route of administration (13). In spite of the structural similarities with drugs in group I and aconitine, drugs in group II did not show any arrhythmogenic activity. On the basis of structure-activity relationship, it is tempting to suggest that the substituent in position 4 must be important for the arrhythmogenic activity. The methylene group in the cyclohexane ring and the oxygen atom does not seem to affect the arrhythmogenic activity since it is also present in aconitine. It would be difficult, however, to do a complete analysis of the structure-activity relationship of these compounds on the basis of the present results. However, due to the structural similarities among these compounds, the therapeutically important possibility may exist that an ap-

parently inactive analogue may block the arrhythmogenic effects of the other substances. A more detailed study would be necessary to prove or disprove this possibility.

ACKNOWLEDGMENTS

This research was supported by NIH research grant HL-12971 awarded by the National Heart, Lung, and Blood Institute, PHS/DHEW. Support from the National Science Foundation towards the purchase of a department nmr instrument is also acknowledged. G.M. was the recipient of a supporting research grant from the Pakistan Science Foundation. The authors are grateful to Professor O. E. Edwards for alkaloidal samples.

Received 23 March 1979.

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