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Implication of C-5, C-6 unsaturation as a key structural factor in steroidal alkaloid-induced mammalian teratogenesis

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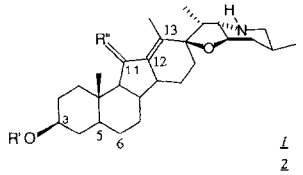
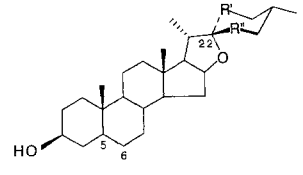
Abstract. Hamster teratogenicity induced upon oral administration of steroidal alkaloids (solanidanes, spirosolanes and jervanes) appears to relate very closely to the presence or absence of C-5, C-6 unsaturation in the alkaloid, which may be more important than molecular configuration at C-22 and placement of the nitrogen atom with regard to the plane of the steroid.
Key words. Solanum alkaloids; Veratrum alkaloids; teratogenesis; craniofacial malformations; hamster.

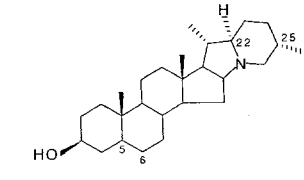
During the early part of this century, epidemics of cyclopia and related congenital malformations in sheep were common in certain high intensity lambing operations in several national forests of Idaho. Defects included single- or double-globe cyclopia and occasionally a skin-covered proboscis above a cyclopic single eye. In the 1960s, oral administration of *Veratrum californicum* on the fourteenth day of gestation was found to induce the disease in the offspring of experimental ewes. Subsequent testing of individual jerveratrum alkaloids revealed that only three were teratogenic: jervine (**1**); 11-deoxojervine (cyclopamine) (**2**); and its 3-glucosyl derivative (cycloposine) (**3**)[1]. Each of the craniofacial, limb, palate, and tracheal terata expressions induced by *Veratrum* in sheep has similar counterparts in humans[2]. The structural similarity between *Veratrum* alkaloid teratogens and certain solanidane and spirosolane *Solanum* alkaloids that occur in human food sources (e.g., potatoes ex *Solanum tuberosum*) inspired numerous investigations[3] of *Solanum* species teratogenicity during the following two decades. However, only a limited number of studies have examined mammalian ingestion of pure compounds. It was suggested that the teratogenicity or lack thereof of isomeric spirosolanes[4] and solanidanes[5] could be related to the position of the alkaloid nitrogen atom relative to the plane of the steroidal ring system. Somewhat later it was reported[6] that either of two major potato alkaloid glycosides, α-solanine or α-chaconine, induced terata in hamsters. The significance to hamster teratogenesis of both the presence of nitrogen in the F-ring of spirosolanes and its configurational placement appeared to be demonstrated by the absence of terata induction by tomatidine (**4**), which bears a nitrogen atom that projects above the steroidal plane and by the non-nitrogenous steroidal sapogenin diosgenin (**5**), in contrast to the teratogenic solasodine (**6**), in which the nitrogen atom is directed below the steroidal plane[4]. Solanidane teratogenicity was proposed in other studies to be dependent upon C-22 configuration because inversion of the natural configuration at this stereocenter produced a synthetic 22*S*,25*R*-solanidane epimer that was much more teratogenic (100% abnormal litters/4 litters) than was 22*R*, 25*S*-5α-solanidan-3β-ol (demissidine) (**7**) (14% abnormal litters/7 litters)[5]. Thus, the assertion by Renwick et al.[6] that potato sprout teratogenicity could be accounted for solely by the sprout content of α-solanine and α-chaconine (presumably of 22*R*,25*S*-configuration[7]) seemed inconsistent with simple stereochemical expectations based upon the teratogenic potency of solanidanes isomeric at C-22.



	R'	R''	C-5,C-6
1	H	O	=
2	H	H_2	=
3	D-Glc	H_2	=
9	H	O	—(5αH);12βH,13αH



	R'	R''	C-5,C-6
4	CH_2	NH	—(5αH)
5	O	CH_2	=
6	NH	CH_2	=
10	NH	CH_2	—(5αH)



	C-5,C-6
7	—(5αH)
8	=

Scheme

Congenital malformations induced by steroidal alkaloids in hamsters*

Substance	Dose# (mg/kg)	Litters (abnormal§/normal)	Percent abnormal litters	Statistical significance between paired compounds (p)†	Percent abnormal fetuses	Statistical significance between paired compounds (p)‡
A. Solanidanes						
22*R*,25*S*-Solanidine (**8**)	176	6/12	50 (0.003)[a]	0.089	24 (<0.0001)[b]	<0.001
22*R*,25*S*-5α-Solanidan-3β-ol (**7**)	176	2/14	14 (0.22)NS[c]		3 (0.17)NS	
0.33% aq. propylene glycol (containing trace EtOH)	—	0/15	0	—	0	—
B. Spirosolanes and jervanes						
Solasodine (**6**)	1400	6/8	75 (0.0004)[a]	0.35	29 (<0.0001)[b]	<0.0002
Dihydrosolasodine (**10**)	1400	5/11	45 (0.009)		6 (0.005)	
Jervine (**1**)	118	10/10	100 (<0.0001)	0.01	92 (<0.0001)	<0.0001
Tetrahydrojervine (**9**)	119	4/10	40 (0.02)NS		14 (<0.0001)	
0.33% aq. propylene glycol (containing trace EtOH)	—	0/14	0	—	0	—

*Dosed on the eighth day of gestation (see *Materials and methods*). #See *Materials and methods* for molar dosage. §Abnormalities observed for **7** were encephalocele; for **8**, exencephaly, encephalocele, anophthalmia and external viscera; for **6** and **9**, encephalocele, exencephaly and anophthalmia; for **10**, encephalocele, cebocephaly, exencephaly and anophthalmia; and for **1**, cebocephaly, exencephaly, anophthalmia and harelip/cleft palate. †Probability values (p) of abnormal litters occurring due to chance were obtained by chi-square analysis using two-tailed Fisher exact results. Values represent a comparison of treatment with the C-5, C-6 unsaturated alkaloid vs. the saturated analog. ‡Similar to (†) except that values are for abnormal fetuses. [a]Numbers in parentheses are Fisher probability values of the occurrence of abnormal litters by comparison of treatment with alkaloid vs. carrier controls. [b]Similar to (a) except that values are for abnormal fetuses. [c]NS = Not significant.

Materials and methods

Solanidine (**8**) [mp 216–217 °C, $[\alpha]_D^{27} - 28.9(CHCl_3)$] was produced upon ethanolic HCl hydrolysis of α-solanine that had been obtained by standard methods from a crude alkaloidal extract which had been isolated[8] from Kennebec variety potato sprouts. The 22*R*,25*S*-configuration of the isolated solanidine (**8**) was confirmed by comparison of its GC/MS with those of its synthetic epimers. 22*R*,25*S*-5α-Solanidan-3β-ol (**7**) [mp 219–220 °C, $[\alpha]_D^{27}$ 29.5 $(CHCl_3)$] and tetrahydrojervine (**9**) [mp 221 °C(dec.)] were obtained by hydrogenation of compounds **8** and **1**, respectively, over PtO_2 in acetic acid. Dihydrosolasodine (**10**) [mp 206–207 °C] was obtained by hydrogenation of compound **6** over Pd on carbon in acetic acid.

Female Syrian hamsters (Simonsen, Gilroy, CA) were administered alkaloid samples by stomach tube on the eighth day of gestation, a period of known susceptibility to craniofacial malformations[9] (see table), employing established protocol.[10]

Alkaloids **7** and **8** were dosed to pregnant hamsters at a constant weight basis of 4.4×10^{-4} M/kg of body weight using 0.33% aq. propylene glycol (3 ml) containing a trace of ethanol as carrier.

Alkaloids **1**, **9**, **6** and **10** were dosed at a constant weight basis of 2.8×10^{-4} M/kg of body weight for the former pair and 3.4×10^{-3} M/kg of body weight for the latter pair. Dosages were selected for each compound pair to ensure an incidence of malformed litters of near 50% for at least one member of the pair. Carrier controls were employed for each test substance.

Results and discussion

In order to determine whether steroidal alkaloid-induced malformations were dependent upon the chirality at C-22, clarification was required of the configurational relationship of the C-22 and C-25 stereocenters of each administered alkaloid compared to its induced teratogenicity. Administration of 22*R*,25*S*-solanidine (**8**) (the aglycone of both α-solanine and α-chaconine) to hamsters by stomach tube induced craniofacial malformations in 50% of the litters from treated dams (table). Thus, terata induction by this naturally occurring solanidane (whose basic nitrogen functionality is directed above the beta face of the steroidal plane) demonstrates that configuration-terata relationships proposed for isomeric spirosolanes based upon projection of the imino group cannot be extrapolated to isomeric solanidanes. 22*R*,25*S*-Solanidanes previously had been deemed non-teratogenic because administration of demissidine (**7**) to seven animals at non-lethal doses induced only one litter containing a single malformed offspring[5]. However, administration of this alkaloid did induce a relatively high fetal resorption rate that was noted with interest because fetal resorption and teratogenicity are often closely related[5]. Reexamination of the teratogenicity of the 5,6-dihydro compound **7** in the present experiments employing a larger number of animals revealed that induction of terata did occur at a level of 14% (abnormal litters) (p = NS compared to controls), which was markedly lower than that observed for solanidine (**8**) (50% abnormal litters, p = 0.003 compared to controls) (table). Comparison of

abnormal fetuses induced by each alkaloid to control fetuses revealed a similar trend (table). Comparison of the statistical difference between incidence of abnormal litters as well as abnormal fetuses induced by the 5,6-dehydro vs. the 5,6-dihydro compound showed p values of 0.089 for litters and less than 0.001 for fetuses (table). Although an absolute structural demarcation between teratogenicity and non-teratogenicity was not observed for these two solanidanes, the pronounced difference in malformation potency observed for 22*R*,25*S*-solanidine (**8**) (50% incidence) vs. 22*R*,25*S*-5α-solanidan-3β-ol (**7**) (14% incidence) focused attention on the significance of their only structural dissimilarity, i.e., the presence or absence of C-5, C-6 unsaturation. It suggested further that the teratogenic differences observed for spirosolanes[4] (tomatidine (**4**) vs. solasodine (**6**)) may also be more closely related to unsaturation at C-5, C-6 rather than to the opposite configuration at C-22 of these alkaloids. A comparison (see table) of the terata induced upon administration of 5,6-dihydrosolasodine (**10**) (45% abnormal litters) with that induced by solasodine (75% abnormal litters) supported this possibility although not statistically significant. The inspection of incidence of terata of individual fetuses induced in dams that had been administered the two spirosolanes was more conclusive and highly significant statistically. 5,6-Dihydrosolasodine induced malformations in only 6% of the derived fetuses whereas solasodine-treated dams showed terata in 29% of their fetuses (table).

The pattern of steroidal alkaloid-induced terata expression[1] is quite different between the spirosolane solasodine (**6**) (primarily brain exposure) on the one hand and that of the 22*S*,25*R*-solanidanes and jerveratrum alkaloids (harelip, cleft palate and cebocephaly in addition to exposed brain) on the other. However, very similar terata are induced by spirosolane **6** and 22*R*,25*S*-solanidine (**7**). This may suggest a potential biological or biochemical mechanistic similarity between the latter pair of alkaloids not shared with the former group, assuming there were similar absorption and clearance rates for all compounds so as to allow identical insult times. Reduction of the ring-D 12,13-double bond in jervine afforded a compound that had been shown[11] to produce no lowering of the potent teratogenicity of jervine. However, in the present experiments, reduction of the ring-B 5,6-double bond yielded tetrahydrojervine (**9**) that was dramatically less teratogenic than either jervine (**1**), 12,13-dihydrojervine[11] or cyclopamine[9] (**2**). Ninety-two percent of the fetuses obtained from animals administered jervine possessed malformations; however, terata were present in only 14% of fetuses derived from tetrahydrojervine-treated animals. The statistical significance of this comparison was highly significant ($p < 0.0001$) (table). There was also a statistically significant difference between number of abnormal litters induced by jervine (100%) compared to abnormal litters induced by tetrahydrojervine (40%) ($p = 0.01$). Thus, steroidal alkaloids that have induced both nasal and brain defects (jervanes) and those that yielded only the latter defects (22*R*,25*S*-solanidanes and spirosolanes) are both less teratogenic upon the removal of unsaturation at C-5, C-6. Mechanisms of bioactivity that might account for terata differences between alkaloids saturated or unsaturated at C-5, C-6 range from potential conversion of the C-5, C-6 bond by epoxidation into an alkylating agent capable of reacting with cellular nucleophiles (cf, ref. 12) to differences in circulatory system absorption (cf, ref. 13).

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