Pure & Appl. Chem., Vol. 66, Nos 10/11, pp. 2407–2410, 1994.
Printed in Great Britain.

Structure–activity relations of teratogenic natural products

William Gaffield and Richard F. Keeler

Western Regional Research Center, ARS, USDA, Albany, CA 94710 and Poisonous Plant Research Laboratory, ARS, USDA, Logan, UT 84321

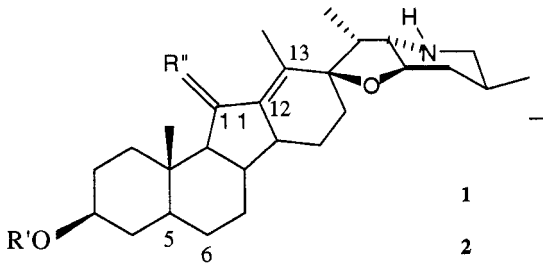
Abstract: Mammalian ingestion of jervane, solanidane, and spirosolane steroidal alkaloids produces craniofacial congenital malformations in offspring upon administration during the primitive streak/neural plate developmental phase. Structure-terata studies have shown that hamster teratogenicity induced by steroidal alkaloids is primarily related to the presence of C-5, C-6 unsaturation and secondarily to the molecular configuration at C-22 (spirosolanes and solanidanes). Teratogenic potencies of jervanes and solanidanes are appreciably higher than those of spirosolanes whereas the potency of jervanes is generally greater than that of solanidanes. The enhanced teratogenicity of functionalized steroidal alkaloids implies that their amphiphilic nature may be important in facilitating their passage of the embryonic membrane.

INTRODUCTION

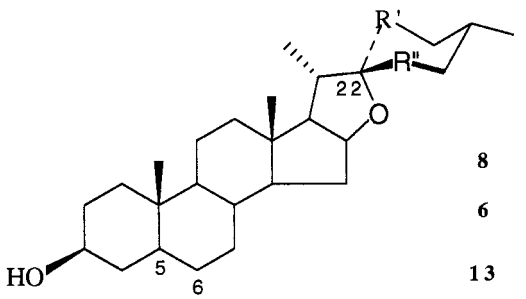
Epidemics of cyclopia and related craniofacial malformations (terata) in newborn lambs were common on sheep ranches in some areas of Idaho during the early part of this century (1,2). A primary expression was known as "monkey-face" lamb disease where animals possessed two closely-spaced corneas in a single distorted sclera. Initially, it was believed that the malformations were due to genetic factors but extensive research revealed that the terata arose when pregnant ewes ingested *Veratrum californicum* on the fourteenth day of gestation (3). Three jerveratrum alkaloids, jervine (1), cyclopamine (2) and cycloposine (3), that were isolated from the plant, induced the disease when orally administered to pregnant ewes during the primitive streak/neural plate stage of embryonic development (4). Other ruminants that were susceptible to *Veratrum* alkaloids included cattle and goats. Experiments on non-ruminants showed that the offspring of mice, rats and hamsters also developed terata upon maternal ingestion of cyclopamine (5). Because each of the craniofacial and other terata expressions induced by *Veratrum* has related counterparts in humans (6), numerous solanidanes and spirosolanes that are present in edible portions of various plants used as human food ((*e.g.*, *Solanum tuberosum* (potato), *Solanum melongena* (eggplant) and *Lycopersicon esculentum* (tomato)) have been evaluated (7) as potential teratogens. Oral administration of solanidanes and spirosolanes to hamsters induces primarily brain defects, in particular, exencephaly (fully exposed brain) and encephalocele (herniation of the brain characterized as a protrusion of meningeal or skin-covered brain tissue). In addition to brain malformations, jervanes induce nasal terata in hamsters such as cebocephaly (a misshapen nasal chamber having an absent or incomplete nasal septum) and cleft palate (7).

STRUCTURE-TERATA RELATIONS

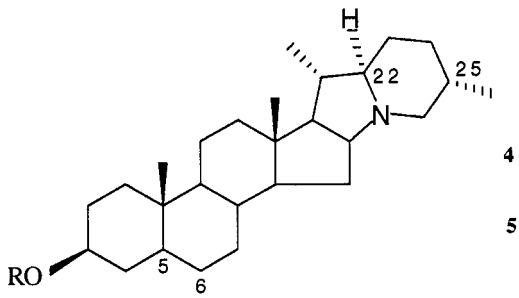
Structurally diverse *Solanum* steroidal alkaloids exhibited varied results in hamsters. Solanidine glycosides α-solanine (4) and α-chaconine (5) both induced (8) craniofacial malformations whereas the spirosolanes tomatidine (6) and tomatine (7) (3-0-ß-lycotetraose-6) were non-teratogenic even at high dosage (9). Solasodine (8) was teratogenic although at a dosage nearly ten-fold that required for terata induction by cyclopamine (9). A synthetic 22*S*, 25*R*-epimer of solanidine displayed teratogenicity similar to that of jervine whereas naturally-occurring 22*R*, 25*S*-solanidanes were thought to be non-teratogenic because of the insignificant incidence of terata induced upon administration of demissidine (9) (10).



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



	R'	R"	C-5,C-6
8	NH	CH_2	═
6	CH_2	NH	— (5αH)
13	NH	CH_2	— (5αH)



	R	C-5,C-6
4	Glc, Rha > Gal	═
5	Rha, Rha > Glc	═
9	H	— (5αH)
11	H	═

Consequently, conformational analysis was used to devise a hypothesis that related induced teratogenicity to a negatively charged center accessible to the *alpha* side of the steroidal plane (10). This correlation was based upon two comparisons in particular; that of the spirosolanes solasodine *vs* tomatidine and of 22*S*, 25*R*-solanidine *vs* 22*R*, 25*S*-dihydrosolanidine (demissidine) (**9**) (10). However, both of these analogies involved the comparison of 5,6-dehydro *vs* 5,6-saturated steroidal alkaloids.

In order to determine whether steroidal alkaloid-induced teratogenicity in hamsters was critically dependent upon the absolute configuration at C-22, induction of terata was measured after administration of jervanes, solanidanes and spirosolanes that differed only in their saturation at C-5, C-6. In all three comparisons, steroidal alkaloids that have induced both brain and nasal malformations (jervanes) and

TABLE 1. Teratogenic potencies of steroidal alkaloids in hamsters[a]

Alkaloid	Teratogenic potency[b]
Jervine (1)	100
12ß, 13α-Dihydrojervine (12)	60
α-Chaconine (5)	50
22*S*, 25*R*-Solanidanes	50
5α, 6, 12ß, 13α-Tetrahydrojervine (10)	40
Cyclopamine (2)	35
Solanidine (11)	35
α-Solanine (4)	35
5α, 6-Dihydrosolanidine (9)	10
Solasodine (8)	6
5α, 6-Dihydrosolasodine (13)	4
Tomatine (7)	1
Tomatidine (6)	0

[a] See text for caveat.

[b] Comparisons derived from data of Brown and Keeler (refs. 10,19,20); Gaffield and Keeler (ref. 11); Gaffield et al. (ref. 21); Keeler *et al.* (refs. 9,22); and Renwick *et al.* (ref. 8).

those that have induced only the former defects (22*R*, 25*S*-solanidanes and spirosolanes) were both significantly less teratogenic upon the removal of C-5, C-6 unsaturation (11). Thus, ninety-two percent of the fetuses obtained from animals administered jervine possessed malformations whereas terata occurred in only 14% of fetuses derived from tetrahydrojervine (10)-dosed animals (11). Similarly, the percentage of deformed fetuses derived from solasodine and solanidine (11)-treated animals diminished from 29 to 6 percent and from 24 to 3 percent, respectively, upon saturation of the C-5, C-6 linkage (11). Clearly, hamster teratogenicity induced upon oral administration of steroidal alkaloids correlates closely with the presence or absence of C-5,C-6 unsaturation in the alkaloid and this factor may be more important in structure-terata relations than molecular configuration at C-22 and placement of the amino group with respect to the steroidal plane (11).

A table of relative teratogenic potencies (using the litter as the experimental unit) of natural and synthetic steroidal alkaloids has been proposed based upon extrapolation of literature data to equivalent oral dosage (Table 1) (12). It has been emphasized that the correlation is only approximate because the literature data were obtained from different controlled animal experiments at two research laboratories using hamsters from the same supplier, but whose relative teratogenic susceptibilities may only in retrospect be assumed to be similar (12). Teratogenic potencies of jervanes and solanidanes are appreciably higher than those of spirosolanes while the potency of jervanes is generally greater than that of solanidanes. Jervine, the most potent steroidal alkaloid teratogen, is the most highly functionalized molecule bearing two oxygenated substituents and two double bonds. Removal of the 11-keto group of jervine yields cyclopamine whose relative teratogenicity parallels that of solanidine. Unlike jervine, cyclopamine undergoes facile ring-E opening at an acid concentration equivalent to that of a non-ruminant stomach to yield the highly toxic but non-teratogenic veratramine (13).

BIOLOGICAL IMPLICATIONS

The structure-terata relations of steroidal alkaloids imply that the presence of functionality such as unsaturation and oxygenated substituents (*cf*, jervine) strongly enhances their teratogenicity (Table 1). This observation focuses attention on the amphiphilic properties of functionalized steroidal alkaloids and leads to speculation that a critical balance of hydrophobicity and hydrophilicity is required to permit passage of the alkaloid teratogen through the placental barrier. After passing the placental barrier, the

teratogens may adversely affect a process or sequence during the primitive streak/neural plate phase that is critical for embryonic growth or development. A subtle change in molecular structure from the solanidane to the jervane ring system appears to induce adverse effects in hamsters not only to brain development but also to nasal passage formation.

Mechanisms for terata induction by jerveratrum alkaloids have been proposed both for craniofacial malformations and for limb defects. Australian researchers suggested that catecholamine-secreting cells in embryonic neuroepithelium are a specific target for the expression of steroidal alkaloid-induced terata (14,15). Thus, jerveratrum alkaloids may competitively bind to the nicotine receptor, an observation consistent with the effect of *Veratrum* teratogens on the embryonic cranial neuroepithelium (16). Shortening of various limb bones induced when pregnant ewes ingest *V. californicum* at gestation periods later than the primitive streak/neural plate phase may result from jervine compromising rapidly developing chondrogenic precursors (17). Prior to differentiation, exposure of limb bud mesenchyme cells to a *Veratrum* teratogen *in vitro* suppressed subsequent accumulation of cartilage proteoglycan (18). Further research on specific biochemical mechanisms that may be inhibited by steroidal alkaloid teratogens is essential to obtain a clearer understanding of their role in mammalian teratogenesis.

REFERENCES

1. R.F. Keeler, in *Isopentenoids in Plants: Biochemistry and Function*, W.D. Nes, G. Fuller and L.-S. Tsai, eds., p. 531, Dekker, New York (1984).
2. R.F. Keeler, in *Alkaloids: Chemical and Biological Perspectives*, Vol. 4, S.W. Pelletier, ed., p. 389, Wiley, New York (1986).
3. W. Binns, J.L. Shupe, R.F. Keeler, and L.F. James, *J. Am. Vet. Med. Assoc.* **147**, 839 (1965).
4. R.F. Keeler and W. Binns, *Teratology* **1**, 5 (1968).
5. R.F. Keeler, *Proc. Soc. Exp. Biol. Med.* **149**, 302 (1975).
6. J. Warkany, *Congenital Malformations*, Year Book Medical Publ., Chicago (1971).
7. R.F. Keeler, D.C. Baker, and W. Gaffield, in *Handbook of Natural Toxins: Toxicology of Plant and Fungal Compounds*, Vol. 6, R.F. Keeler and A.T. Tu, eds., p. 83, Dekker, New York (1991).
8. J.H. Renwick, W.D.B. Claringbold, M.E. Earthy, J.D. Few, and C.S. McLean, *Teratology* **30**, 371 (1984).
9. R.F. Keeler, S.Young, and D. Brown, *Res. Commun. Chem. Pathol.Pharmacol.* **13**, 723 (1976).
10. D. Brown and R.F. Keeler, *J. Agric. Food Chem.* **26**, 566 (1978).
11. W. Gaffield and R.F. Keeler, *Experientia* **49**, 922 (1993).
12. W. Gaffield and R.F. Keeler, in *Poisonous Plants of the World: Phytochemical, Agricultural and Ecological Aspects*, S.M. Colegate, P.R. Dorling, and K.E. Panter, eds., CAB Press, Cambridge, UK (in press).
13. D. Brown,in *Effects of Poisonous Plants on Livestock*, R.F. Keeler, K.R. Van Kampen, and L.F. James, eds, p. 409, Academic Press, New York (1978).
14. F.R.P. Sim, B.G. Livett, C.A. Browne, S.E. Moore, R.F. Keeler, and G.D. Thorburn, *Teratology* **30**, 50A (1984).
15. F.R.P. Sim, B.G. Livett, C.A. Browne, and R.F. Keeler, in *Plant Toxicology*, A.A. Seawright, M.P. Hegarty, L.F. James, and R.F. Keeler, eds., p. 344, Queensland Poisonous Plants Comm., Yeerongpilly, Australia (1985).
16. F.R.P. Sim, N. Matsumoto, E.H. Goulding, K.H. Denny, J. Lamb, R.F. Keeler, and R.M. Pratt, *Teratog. Carcinog. Mutagen.* **3**, 111 (1983).
17. M. Campbell, W. Horton, and R.F. Keeler, *Teratology* **36**, 235 (1987).
18. M. Campbell, K.S. Brown, B. Saunders, J. Hassell, E. Horigan, and R.F. Keeler, *Teratology* **29**, 22A (1984).
19. D. Brown and R.F. Keeler, *J. Agric. Food Chem.* **26**, 561 (1978).
20. D. Brown and R.F. Keeler, *J. Agric. Food Chem.* **26**, 564 (1978).
21. W. Gaffield, R.F. Keeler, D.C. Baker, and A.E. Stafford, in *Poisonous Plants*, L.F. James, R.F. Keeler, E.M. Bailey, Jr., P.R. Cheeke, and M.P. Hegarty, eds., p. 418, Iowa State Univ. Press, Ames, IA (1992).
22. R.F. Keeler, D.C. Baker, and W. Gaffield, *Toxicon* **28**, 873 (1990).