Structure-Activity Relation of Steroid Teratogens. 1. Jervine Ring System

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Derivatives of jervine and 11-deoxojervine have been synthesized with differing functionality in the steroid A, B, C, and D rings to test the relation of structure to activity of steroid teratogens in hamsters. Two other compounds, muldamine and 11-deacetylmuldamine, were tested to determine the importance of a rigid side chain in the molecule. The relative activities of these compounds suggest that they act as steroid hormone blocking agents in the developing embryo. It is concluded that the atypical steroid ring system of jervine spirofuranopiperidine alkaloids is not crucial to activity and the rigid side chain of these alkaloids may only enhance teratogenicity. Further, conventional steroids having a basic imino group accessible to the α face of the D ring would have a probability of substantial teratogenicity.

Two veratrum alkaloids of the spirofuranopiperidine type, jervine (1, Figure 1) (Kupchan and Suffness, 1968) and 11-deoxojervine (2, Figure 1) (Masamune et al., 1965; Kupchan and Suffness, 1968) from false hellebore (*Veratrum californicum* Durand) cause congenital defects in a variety of mammals (Keeler, 1975). The 3-glycoside of 2, cycloposine (Keeler, 1969) also found in the plant is teratogenic in sheep. Defects may be caused by oral dose during approximately the primitive streak/neural plate stages of embryonic differentiation and are largely osteocranial, although sheep fed 2 during week 5 of gestation have given rise to offspring with limb deformities (Keeler, 1973). The atypical, highly functionalized structure of jervine teratogens has prompted investigating derivatives of these alkaloids. The purpose being to determine if the parent compounds have exclusive properties requisite to this activity.

Little is known about the relation of activity to structure of steroid alkaloid teratogens. The 11-keto group of 1 is not needed for activity as compound 2 is at least equally active. Loss of the 11-keto function does, however, affect activity in another way. Compound 2 is very susceptible to electrophilic attack and subsequent opening of the ether bridge (E ring). Stomach acid in the rabbit is reported able to cause 2 to open ring E and thus reduce activity (Keeler, 1970a). A similar reaction of 1 requires more drastic conditions presumably because the ether bridge is stabilized by the conjugated 11-keto group. Related alkaloids lacking the ether bridge (e.g., veratramine) have been reported inactive in sheep (Keeler, 1970b).

Evidence that the nitrogen is crucial to the activity of these alkaloids includes (in addition to the above suggestion that a rigid furanopiperidine system may be required) recent findings on the activity of compounds related to the solanum spirosolane group alkaloids (Keeler et al., 1976). Solasodine (3, Figure 1) was reported about one-tenth as active as veratrum teratogens while tomatidine (4, Figure 1) and diosgenin (5, Figure 1) failed to produce abnormalities when fed at even greater dose levels than 3. These data support the conclusion that the nitrogen is relevant to activity and that the function apparently must be accessible to the α face of the steroid plane. The relatively low activity, and inactivity respectively, of 3 and 4 could, however, reflect only differences in basicity shown in Table I. Note that although they retain substantial basicity, 3 and 4 are not strictly amines, but spiroketimines. The spiroketal containing saponin 5 is relatively nonbasic. Other parameters involved in the activity of jervines must relate to steric and/or functional factors associated with the atypical steroid skeleton of the active veratrum alkaloids.

Table I. pK_a Values of Steroid Alkaloids

Alkaloid	pK_a[a]
1, jervine	7.40
3, solasodine	7.04
4, tomatidine	6.50

[a] Holubek and Strouf (1965).

This paper gives activity data in the hamster on derivatives of 1 and 2 differing in the steroid portion of the molecule. All of the functional groups in the steroid A, B, C, and D rings were either altered or eliminated. Two related compounds muldamine and deacetylmuldamine (6 and 7, Figure 1) lacking the ether bridge in ring E (Keeler, 1971) were also tested to assess the relative importance of a rigid furanopiperidine system to activity.

MATERIALS AND METHODS

Materials and Apparatus. Jervine used was from S. B. Penick & Co., but is not currently available from that source; 11-deoxojervine and muldamine were isolated from *V. californicum* by an existing method (Keeler, 1973). Mass spectral (MS), IR, and NMR analyses were obtained on Hitachi Perkin-Elmer RMS-4, Beckman IR-4, and Perkin-Elmer R-12B spectrometers, respectively. The NMR solvent was deuteriochloroform with 3% tetramethylsilane as an internal standard. MS samples were inserted by heated probe. Synthesized compounds were tested for homogeneity by TLC on silica gel (Merck AG) in solvent systems of appropriate polarity, and melting points were compared to literature values. All physical data were consistent with assigned structures. Syrian Golden hamsters were supplied by Simonsen Laboratories (Gilroy, Calif.) and Engle Laboratory (Farmersburg, Ind.). Engle supplied timed-pregnancy hamsters for a small portion of this study.

All of the derivatives tested have been reported. 12β,13α-Dihydrojervine was synthesized by catalytic hydrogenation in 0.1 M base (Anliker et al., 1952; Masamune et al., 1969). 3-*O*-Acetyljervine was prepared by solvolyzing 1 in refluxing acetic acid (Iselin et al., 1956). The 11-keto group of the above dihydrojervine was reduced with $LiAlH_4$ to the 11α-ol and by sodium in butanol to the 11β-ol (Masamune et al., 1967). 11-Deoxojervine-4-en-3-one was prepared by Oppenauer oxidation of 11-

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0021-8561/78/1426-0561$01.00/0

Table II. Abnormal and Resorbed Offspring in Hamsters Fed Jervine and Related Compounds

	Dose,[a] mg/kg	Dams fed	Dams 100% resorbed	Resorption, %	Overdose death	Abnormal Offspring, %	Abnormal Litters, %	P[c]	Mean litter
12β,13α-Dihydrojervine	150	5	0	64	4	100	100		6.00
	113	6	0	18	1	63	60	<0.0005	9.20
	75	5	0	9	1	26	25		8.75
12β,13α-Dihydrojervine-11α-ol	150	17	0	2	3	2	21	<0.01	13.2
	113	4	0	25	0	0	0		11.0
12β,13α-Dihydrojervine-11β-ol	150	22	1	10	3	0	0		11.4
3-*O*-Acetyljervine	225	3	0	77	0	100	100	<0.0005	3.00
	150	11	1	35	0	30	50		6.27
11-Deoxojervine-4-en-3-one	150	17	6	52	7	52	83		5.60
	75	7	0	15	0	59	86		10.1
	38	7	1	19	0	70	83	<0.0005	7.71
	15	9	0	3	0	0	0		6.44
Muldamine	600	11	0	38	2	16	29	<0.05	6.44
	300	4	0	5	0	0	0		9.75
Deacetylmuldamine	150	10	0	16	0	2	20	<0.05	9.90
Jervine[b]	170	8	0	16	1	65	100	<0.0005	10.7
	85	6		0	0	4	20	<0.50	11.4
	42	3		7	0	0	0		9.00
Water[b]		135	1	6.4		0.21	2.2		10.4
Nonfed[b]		57	2	7.3		0.36	3.6		9.96

[a] Suspended in 4 mL of water and fed by stomach tube on day 7 or 8 of gestation. [b] Data from Keeler (1975). [c] Probability of abnormal litters occurring due to chance.

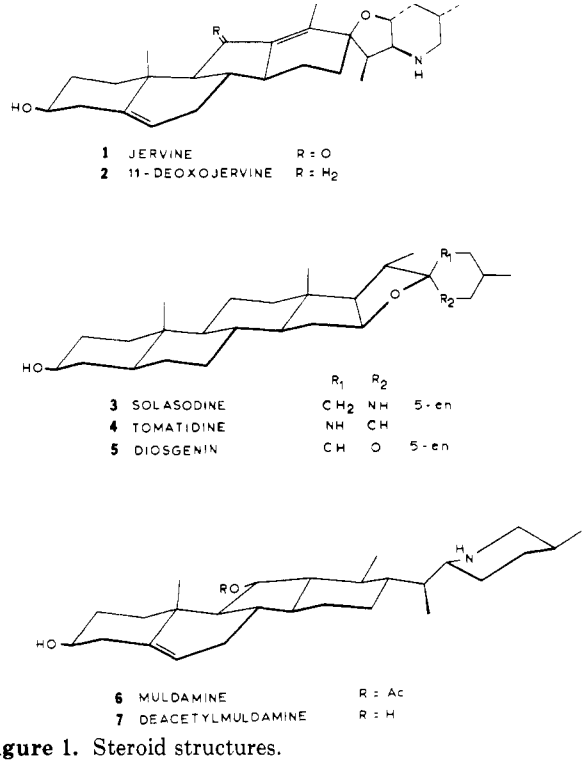

Figure 1. Steroid structures.

deoxojervine (Masamune et al., 1967; Eastham and Teranishi, 1963). 11-Deacetylmuldamine (Keeler, 1971) was prepared by hydrolysis of muldamine in 5% methanolic potassium carbonate at ambient temperature. The crude product contained a small amount of material thought to be the 9(11)-en by MS. It was not characterized fully.

Bioassay. Individually housed, weighed, pregnant hamsters were administered by stomach tube test compounds suspended in 4 mL of water on day 7 or 8 of gestation. Doses were based on a mean animal weight of 132.9 ± 17 g. Hamsters were killed on gestation day 15 and fetuses were examined grossly for abnormalities. Fetuses with more than one abnormality were counted as a single abnormal offspring. Statistical tests were based on a χ^2 distribution of the contingency that abnormal litters in treated groups were due to chance relative to controls. Some dose levels were combined to give larger cells for statistical assay. Resorption percentages were weighted against offspring totals plus resorptions and added with animals that resorbed all offspring. Resorptions were not considered in the total and percentages of abnormal fetuses and were not analyzed statistically.

RESULTS AND DISCUSSION

Results of the animal feeding experiments are given in Table II. The abnormalities encountered in order of decreasing frequency were: harelip and/or cleft palate, cebocephaly, cranial bleb, exencephaly, and microphthalmia. No significant change in the types of abnormality were noted between compounds. A full breakdown of jervine and spirosolane induced malformations is available (Keeler, 1975; Keeler et al., 1976).

The most orally active compound (produced in these experiments) was 11-deoxojervine-4-en-3-one, which appeared to be more than twice as active as jervine on a dose–response basis. Hydrogenating the jervine 12,13 bond or acylating the 3-hydroxyl appeared to have no measurable effect on activity. The two 11-hydroxyl derivatives had reduced but different activities. The 11β-ol did not give rise to abnormal offspring, while the 11α-ol gave rise to a low incidence of abnormal offspring and was apparently as toxic as its epimer. Results with muldamine and deacetylmuldamine (**6** and **7**, Figure 1) suggest that the rigid furanopiperidine system is not necessarily crucial to activity. Further testing of muldamine derivatives lacking the 11α-ol is needed to determine if reduced activity is associated with free rotation of the piperidine-containing side chain or the presence of an 11α-hydroxyl group. Nevertheless, the persistent activity of both compounds coupled with increased activity of the deacetyl product tends to suggest that the 11-substituent is more important to reduced activity than free rotation of the side chain.

The substantial activity of 12β,13α-dihydrojervine (Masamune et al., 1969) is of interest also from a structural standpoint. The jervine D ring can be predicted to be relatively flexible because it is six membered and has a double bond as pictured in Figure 2. This allows some side chain movement perpendicular to the plane of the

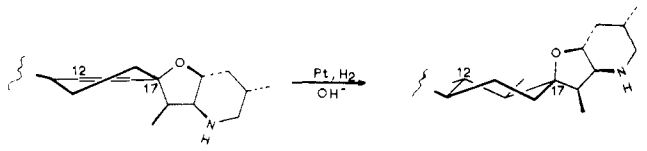

Figure 2. The hydrogenation stereochemistry of the jervine C-12 double bond.

steroid ring system and for that reason does not allow a prediction of the optimum geometry of the nitrogen for activity. The D ring of the dihydro compound is in a rigid chair form with the C–D ring juncture trans and the side chain positioned such that the nitrogen is inclined away from the steroid α face (Figure 2). The retention of substantial activity by this derivative indicates that such a side chain position (and, thus, the imino group) does not alter activity.

The sum of these observations supports a suggestion (Keeler, 1670b) that veratrum teratogens inhibit hormone function in the developing embryo. Primary evidence for this conclusion is the substantially increased activity of 11-deoxojervine-4-en-3-one. Because of its hormone-like A–B ring system, this compound might be expected to reach hormone receptors and interact to a greater degree with hormone sites thus causing more defects.

Results with the 11-hydroxy jervines may also support this conclusion. Additional hydroxyl groups in the steroid nucleus invariably reduce hormone activity (Bush, 1962), presumably by reducing specificity for a receptor site. Further, it is thought that hormone sites bind hydrophobically with the β face of the C ring (Wolff et al., 1964; Korolkovas, 1970). Thus a compound with an 11β-hydroxyl group might be expected to interact to a lesser degree with such a site than a similar compound having an 11α-hydroxyl group. Data on the 11-hydroxyl epimers show that the 11β-ol is not appreciably teratogenic at the administered level while the 11α-ol possibly retained a low potential for causing defects. Muldamine (**6**, Figure 1) which has a bulky 11α-acetoxy group, required a considerably increased dose level but showed an incidence of defects also. Presumably the bulk effect of this substituent is more important than reduced polarity of the 11-acetoxy group (relative to an α hydroxyl group).

Although the difference in activity of **3** and **4** can be attributed to their relative basicities, their activities also may suggest that these compounds act as hormone inhibitors. It is thought that hormones interact with an active site α to the D ring (Korolkovas, 1970; Wolff et al., 1964) thus **3** would be free to bind while **4** would not.

Both estrogens and progestins are synthesized in the placenta (Kellie, 1971), and the present data do not suggest which type hormone may be implicated. It is, however, logical to assume these compounds function by blocking estrogenic sites because of the great variety of substances known to have estrogenic activity. Nevertheless, if the above conclusions are accurate, conventional steroids with a secondary basic nitrogen bonded in a position analogous to hormone binding sites (accessible to the α face of the steroid) would be predicted to be substantially teratogenic.

ACKNOWLEDGMENT

The authors thank R. C. Anderson and R. V. Canfield for discussion. L. M. Dunning and F. D. Bowen provided technical assistance.

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Received for review September 6, 1977. Accepted December 23, 1977. Presented in part at the American Chemical Society Rocky Mountain Regional Meeting, Laramie, Wyoming, June 17–19, 1976. Funding was by specific obligation cooperative agreement (USDA–ARS No. 12-14-5001-75) between the U.S. Department of Agriculture, Agricultural Research Service and the Utah State Agricultural Experiment Station; and an interagency agreement between the U.S. Department of Agriculture, Agricultural Research Service and the National Institutes of Health, National Institute of General Medical Services, as funded by the latter. Mention of commercial products does not constitute endorsement by the U.S. Department of Agriculture, Agricultural Research Service.