Structure-Activity Relation of Steroid Teratogens. 2. N-Substituted Jervines

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Several N-substituted jervine derivatives were synthesized to determine the importance of a secondary nitrogen and its basicity to teratogenicity in mammals. Nitrogen substituent effects were, in order of decreasing teratogenicity: CHO ≃ H ≃ CH_3 > *n*-Bu > Ac > $(CH_3)_2I^-$. This order indicates that a tertiary amino group has about the same potential for activity as the imino group of the parent alkaloid and that both steric and electronic factors are important at the nitrogen. The strongly teratogenic formyl derivative may be the result of either decomposition to the parent alkaloid or the formyl carbonyl acting as the anionic center.

The suggestion that fungus infected (blighted) potatoes were responsible for abnormal incidence of congenital defects (Renwick, 1972) has attracted considerable research interest (Kuć, 1975; Symposium, 1973). Both "solanine" and mixed alkaloid extracts from tubers blighted by *Phytophthora infestans* (Mont.) dBy. or from healthy tubers were reported teratogenic in a chick embryo assay (Mun et al., 1975; Jelínek et al., 1976). However, a third study (Nishie et al., 1975) indicated α-solanine and α-chaconine (two glycosides of the principal potato alkaloid, solanidine) did not produce defects in a similar assay.

Testing in mammals had likewise been inconclusive. Potato preparations were found active in marmosets (Poswillo, 1972), but further testing failed to support this finding (Poswillo, 1973). These tests, however, did not include analysis of the alkaloid content of the preparation which may be an important variable. A substantial body of data indicates that blighted potatoes, mixed alkaloid preparations, and pure potato alkaloids are not active in laboratory rodents (Keeler, 1975; Bell et al., 1976; Chaube and Swinyard, 1976). Abnormal rates of fetal death and resorption were usually observed. Recently, a potato-sprout preparation containing at least 50 times the alkaloid concentration of tubers was reported active in hamsters (Keeler et al., 1976a). Current thinking (Kuć, 1975) suggests that the harmful effects, whether toxic or teratogenic, of potato preparations can be attributed to their alkaloid content. Interestingly, it was recently reported that the potato fungus synthesizes glycosides of solanidine on synthetic media (Maas et al., 1977). Nevertheless, there does not seem to be unequivocal evidence that solanidine, or any other substance from potatoes, can cause birth defects [the alkaloid's toxicity has been recognized for many years (Jadhav and Salunkhe, 1975)].

At present, three steroid alkaloids (excluding glycosides) are known to cause birth defects in mammals (Keeler, 1975; Seifulla et al., 1972). Research has indicated that nitrogen is important to activity (Brown and Keeler, 1978, Keeler et al., 1976b) and to the present there are no steroids with a tertiary nitrogen, as for example, solanidine, reportedly able to cause birth defects in mammals. This research tested the dependence of activity on a secondary nitrogen in known teratogens and the possible additional requirement that the nitrogen be part of a strongly basic function. Simple N-alkyl and N-acyl derivatives of the known teratogen, jervine were tested in hamsters.

MATERIALS AND METHODS

Chemical and Apparatus. The supplies and analytical instrumentation were previously described (Brown and Keeler, 1978b).

Syntheses. Two of the compounds tested have not been previously reported. They were synthesized by routine procedures listed below. One additional compound was prepared by a procedure differing from the literature method.

***N*-Formyljervine.** Jervine (1.00 g, 2.34 mmol) was added to 25 mL of ice-cold 88% formic acid. The stirred mixture was allowed to warm to room temperature for 3 h and diluted with ice water and adjusted to pH 8. The solid was collected with suction and washed with cold water. Recrystallizing twice from boiling methanol gave 0.55 g of *N*-formyljervine: yield 51%: mp 227–230 °C; IR (KBr) 3400 (OH), 1730 (C=O), and 1630 (NCH=O) cm^{-1}; mass spectrum (70 eV) *m/e* (assignment), 453 (M^+), 438 (M – CH_3), 437, 424 (M – CHO); NMR ($CDCl_3$) δ 1.04 (19, 21, 23, CH_3), 2.15 (18, CH_3), 8.07 and 8.50 (s, NCHO).

***N*-Butyl-3-*O*-acetyl-12β,13α-dihydrojervine.** Butyric anhydride (10 mL) was added at room temperature to 12β,13α-dihydrojervine (Anliker et al., 1952) prepared from 2.00 g (4.70 mmol) of jervine dissolved in 10 mL of dry distilled pyridine. After standing 18 h, the mixture, diluted with water, was extracted into chloroform, washed with dilute hydrochloric acid and sodium bicarbonate solutions, dried over sodium sulfate, and concentrated to an oil. The oil was diluted with 50 mL of hexane, let stand a few hours, and refrigerated overnight. The crystalline solid (2.125 g, 80%) was carried forward without additional purification; IR (KBr) 1730 (C=O), 1630 (NC=O), and 3400 (no absorption) cm^{-1}.

The amide was reduced by a reported method (Uhle et al., 1960). The diacyl product dissolved in 75 mL of tetrahydrofuran (THF) was added with cooling and mechanical stirring over a 2-h period to a suspension of 2.1 g (3.75 mmol) of lithium aluminum hydride (LAH) in 20 mL of TFH, in a dry system over N_2. After addition, the mixture was refluxed gently for 3 h. On cooling 4 mL of acetone was added slowly followed by cautious addition of 2.5 mL of water. The suspension was filtered and concentrated, water was added, and the product was collected on a filter. Recrystallizing from aqueous methanol gave 1.64 g of white solid, mp 234–236.5 °C; IR (KBr) 3400 (OH), 1730–1620 (no absorptions) cm^{-1}.

Protection of the 3-ol. The 3-hydroxyl group was protected (Iselin et al., 1956). The above product (1.51 g) was refluxed 18 h in 30 mL of glacial acetic acid. The mixture was diluted with ice water, made up to pH 8, extracted into chloroform, washed with 2% sodium sulfate

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Table I. Data on Hamsters Fed Jervine Derivatives and Reference Compounds

	Dose,[a] mg/kg	Dams fed	Dams 100% resorbed	Resorption, %	Overdose death	Abnormal Offspring, %	Abnormal Litters, %	P[c]	Mean litter
N-Methyljervine	170	18	0	6	0	10	44	<0.0005	9.61
N-Butyl-3-*O*-acetyl-12β,13α-dihydrojervine	170	10	0	10	1	4	22	<0.05	11.9
N-Formyljervine	170	9	0	9	6	7	33	<0.0005	9.67
	85	11	0	9	1	12	60		10.4
N-Acetyljervine	170	27	0	7	0	<1	4		10.9
N,O-Diacetyljervine	170	9	0	1	0	1	11		12.2
N-Methyljervine methiodide	225	5	0	27	0	0	0		7.00
	170	12	0	4	0	<1	8		10.7
Jervine[b]	170	8	0	16	1	65	100	<0.0005	10.7
	85	6	0	0	0	4	20	<0.50	11.4
	42	3	0	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.

solution, and dried over sodium sulfate. The mixture, concentrated and recrystallized from benzene–hexane, gave 620 mg: mp 139–141 °C; IR (KBr) 3400 (OH), 1730 (C=O), 1250 (CO) cm^{-1}.

Oxidation of the 11β-ol (Djerassi, 1956). Jones reagent (8 N chromic acid), 0.6 mL, was added dropwise to 500 mg of the above product in 75 mL of acetone at 15 °C. After 10 min additional stirring, the product was decanted, diluted with water, extracted into chloroform, washed with 2% bicarbonate, and dried over sodium sulfate. The oil remaining after solvent removal crystallized on addition of acetone. The yield was 360 mg (15%) overall: mp 274–277 °C; IR (KBr) 3400 (no absorption), 1730, and 1250 (C=O) cm^{-1}; mass spectrum (70 eV) *m/e* 180 and 149; NMR ($CDCl_3$) δ 2.03 (3-OAc). On TLC the oxidation product gave a small trace corresponding to the acylated intermediate and a more polar major component.

***N*-Methyjervine and *N*-Methyljervine Methiodide.** Attempts to prepare the former compound by a reported method (Saito et al., 1936) yielded only the methiodide salt. *N*-Methyljervine was prepared by a procedure used for methylation of veratramine (Uhle et al., 1960).

***N*-Acetyl Jervines.** *N*-Acetyl and *N,O*-diacetyl jervines were synthesized by a conventional method (Iselin et al., 1956).

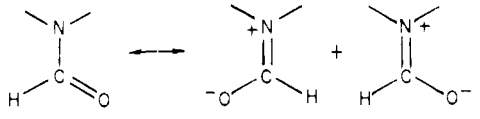
Bioassay. Biological testing was as previously described (Brown and Keeler, 1978b).

RESULTS AND DISCUSSION

Table I gives results of the hamster feeding experiments. The table includes data from jervine feedings in hamsters as a reference of activity. In order of decreasing frequency the following abnormalities were produced: harelip and/or cleft palate, cebocephaly, cranial bleb, exencephaly, and microphthalmia. The incidence of defects did not change materially between compounds tested.

N-Methyljervine retained substantial activity relative to the parent alkaloid, and the *N*-butyl derivative was also marginally active. These results indicate that a tertiary amine retains a substantial potential for causing birth defects and that bulky *N*-alkyl groups, such as the *N*-butyl, reduce the activity to a large degree. These data also suggest that the nitrogen is involved in binding at the active site, probably as an electron-pair donor. Note that the quaternary salt of jervine (without nonbonding electrons) is not significantly active. The strong relationship between the state of the nitrogen atom and activity was previously reported on a less active alkaloid (Keeler et al., 1976b). The data also show that the basicity of the nitrogen was related to activity; both the *N*-acetyl and the quaternary methiodide derivatives retained no significant activity, whereas the strongly basic *N*-methyl derivative was similarly active to the parent alkaloid. The substantially reduced activity of *N*-butyl derivative suggests only steric problems related to the bulk of the alkyl chain.

The relatively high teratogenicity of *N*-formyljervine can be reconciled in two ways. The formyl group may allow the compound to be absorbed more efficiently and subsequent hydrolysis could produce the active species (parent alkaloid). Or a different binding site may function in this compound, where the oxygen of the formyl group functions as the anionic center shown below.



Rotationally nonequivalent isomers of an *N*-formyl steroid alkaloid have been resolved (Toldy and Radics, 1966). Although *N*-formyljervine was not chemically resolved, the NMR spectrum indicates that the formyl proton exists in two rotational states (δ 8.04 and 8.50), suggesting that a strongly anionic carbonyl oxygen contributes substantially to this structure. It cannot at present be rejected that the formyl derivative is the active substance in this instance in spite of the inactivity of *N*-acetyl jervines.

Finally it is possible to rule out the notion that a secondary amino group is necessary to the potential for causing birth defects. Under these conditions, it is entirely possible that solanidine and its glycosides could have a finite potential for causing neural tube closure problems during embryonic differentiation.

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Structure–Activity Relation of Steroid Teratogens. 3. Solanidan Epimers

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Three C-22, C-25 solanidan epimers were tested for teratogenicity in hamsters on oral administration during embryonic differentiation. Both (22*S*,25*R*)-solanid-5-en-3β-ol and (22*S*,25*R*)-5α-solanidan-3β-ol were at least as active as highly teratogenic jervine alkaloids. The other two epimers (22*S*,25*S*)-5α-solanidan-3β-ol and (22*R*,25*S*)-5α-solanidan-3β-ol (the usual natural configuration) primarily caused increased resorption. The activities of compounds with the three configurations were explained by the conformation of the nitrogen. The highly teratogenic compounds present an unhindered nitrogen nonbonding electron pair accessible to the α steroid face.

Solasodine (1, Figure 1), a spirosolane member of the *Solanum* group of alkaloids, was orally teratogenic in rats (Seifulla and Ryzhova, 1972) and hamsters (Keeler et al., 1976a) when fed during embryonic differentiation. The latter report additionally proposed that a basic nitrogen accessible to the steroid α face was a structural requirement for activity. This hypothesis was based on the inactivity of tomatidine (2, Figure 1) at twice the solasodine dose. Solasodine, with a conventional steroid system, was, however, only about one-tenth as active in the hamster as teratogenic jervine alkaloids (3 and 4, Figure 1). This comparatively low activity has left a number of questions about the relation of structure to activity in steroid alkaloids and particularly the importance of the unconventional jervine ring system to activity, as well as the proposed configurational requirements.

To further test these requirements we sought a conventional steroid amine as active as jervine in a group of epimeric solanidans (Sato and Ikekawa, 1961). These compounds are also significant because recent reports (Mun et. al., 1975; Jelinek et al., 1976) link the usual naturally occurring epimer (22*R*,25*S*)-solanid-5-en-3β-ol (solanidine, 5, Figure 1) to birth defects in the chick embryo. Additionally, a potato preparation with a high content of the same alkaloid was active in the hamster (Keeler et al., 1976b). The question of whether potatoes induce birth defects in humans has been investigated extensively (Kuč, 1975). Although the suspicion that the potato alkaloid may pose a teratogenic hazard for humans persists, no direct evidence that a compound of the solanidine structure might cause birth defects has been found. This report gives data on compounds closely related to the potato alkaloid, supporting the proposed structural requirements for teratogenic activity. It is the continuation of a series on this subject (Brown and Keeler, 1978).

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MATERIALS AND METHODS

Chemicals and Apparatus. Solasodine was obtained from Steraloids Inc. (Wilton, N.Y.) and tomatidine from ICN Pharmaceuticals Inc. (Cleveland, Ohio). The instrumentation used in testing synthesized compounds was previously reported (Brown and Keeler, 1978). Products occurring as a mixture of two epimers were separated by column chromatography on neutral aluminia (Woelm Activity Grade II) using a benzene/ethyl acetate:benzene (1:1) linear gradient for elution. The IR analysis of the 2700–3000 cm^{-1} region was performed on a dilute carbon tetrachloride solution using a Perkin-Elmer 281 spectrometer. Timed-pregnancy Syrian Golden hamsters were supplied by Engle Laboratory (Farmersburg, Ind.).

Synthesis. The known method (Sato and Ikekawa, 1961) was modified and used as in the following synthesis