Djerassi, C., Engle, R. R., Bowers, A., *J. Org. Chem.* **21**, 1547 (1956).
Iselin, B. M., Moore, M., Wintersteiner, O., *J. Am. Chem. Soc.* 78, 403 (1956).
Jadhav, S. J., Salunkhe, D. K., *Adv. Food Res.* **21**, 307 (1975).
Jelínek, R., Kyzlink, V., Blattný, C, Jr., *Teratology* **14**, 335 (1976).
Keeler, R. F., *Proc. Soc. Exp. Biol. Med.* **149**, 302 (1975).
Keeler, R. F., Brown, D., Douglas, D. R., Stallknecht, G. F., Young, S., *Bull. Environ. Contam. Toxicol.* **15**, 522 (1976a).
Keeler, R. F., Young, S., Brown, D., *Res. Commun. Chem. Pathol. Pharmacol.* **13**, 723 (1976b).
Kuč, J., *Recent Adv. Phytochem.* **9**, 139 (1975).
Maas, M. R., Post, F. J., Salunkhe, D. K., 1977 Intermountain Branch Meeting, American Society for Microbiology, Idaho State University, Pocatello, Idaho, Mar 25–26, 1977.
Mun, A. M., Barden, E. S., Wilson, J. M., Hogan, J. M., *Teratology* **11**, 73 (1975).
Nishie, K., Norred, W. P., Swain, A. P., *Res. Commun. Chem. Pathol. Pharmacol.* **12**, 657 (1975).
Poswillo, D. E., Sopher, D., Mitchell, S. J., *Nature (London)* **239**, 462 (1972).
Poswillo, D. E., Sopher, D., Mitchell, S. J., Coxon, D. T., Curtis, R. F., Price, K. R., *Teratology* **8**, 339 (1973).
Renwick, J. H., *Brit. J. Prev. Soc. Med.* **26**, 67 (1972).
Saito, K., Suginome, H., Takaoka, M., *Bull. Chem. Soc. Jpn.* **11**, 172 (1936).
Seifulla, Kh. I, Ryzhova, K. E., *Mater. Vses. Konf. Issled. Lek. Rast. Perspekt. Ikh Ispol'z. Proizvod. Lek. Prep.* **1970**, 160 (1972); *Chem. Abstr.* **83**, 22383t (1975).
Symposium, "Potatoes and Birth Defects", *Teratology* **8**, 317 (1973).
Toldy, L., Radics, L., *Tetrahedron Lett.*, 4753 (1966).
Uhle, F. C., Krueger, J. E., Sallmann, F., *J. Am. Chem. Soc.* **82**, 489 (1960).

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

Dennis Brown* and Richard F. Keeler

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

Table I. Abnormal and Resorbed Offspring in Hamsters Fed Solanidan Epimers on Day 8 of Gestation

	Dose,[a] mg/kg	Dams fed	Dams 100% resorbed	Resorption, %	Overdose death	Abnormal Offspring, %	Abnormal Litters, %	Mean litter
(22*R*,25*S*)-5α-Solanidan-3β-ol	343	4	2	67	1	0	0	8.00
	187	3	0	19	0	0	0	8.67
	150	4	0	20	0	3	25	8.25
(22*S*,25*S*)-5α-Solanidan-3β-ol	312	3	1	67	0	0	0	3.33
	165	3	0	3	1	0	0	14.5
(22*S*,25*R*)-5α-Solanidan-3β-ol	177	3	3	100	1	0	0	0.00
	81	4	0	19	0	25	50	8.00
	42	4	0	0	0	0	0	9.00
(22*S*,25*R*)-Solanid-5-en-3β-ol	184	4	0	75	0	75	100	4.00
	88	4	0	25	0	42	50	6.00
	39	4	0	0	0	8	25	9.75
Jervine	127	25	0	7.3	1	16	36	5.08
Water		42	0	3.9	0	0.3	2	9.45

[a] Suspended in 4 mL of water and fed by stomach tube.

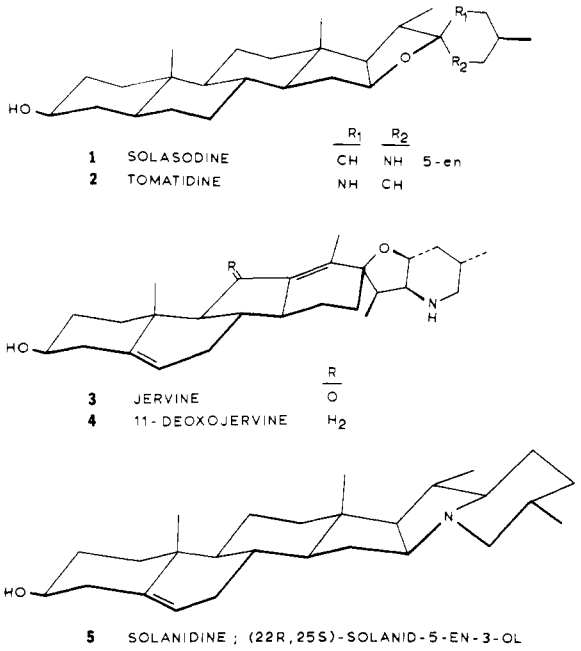

Figure 1. Steroid alkaloid structures.

of (22*S*,25*R*)-solanid-5-en-3β-ol. 3-*O*-Acetyl-16,22-dihydrosolasodine (Kusano et al., 1970) (1.00 g, 2.19 mmol) was dissolved in 5 mL of acetic acid and diluted with 175 mL of acetone. Aqueous chromic acid (8 N) (0.55 mL) was added dropwise at 15 °C over a 10-min period with stirring. The mixture was allowed to warm to ambient for 30 min additional and decanted into 800 mL of ice and water. Sodium hydroxide (4 M) was added to pH 8 and the precipitate was collected with suction, washed with cold water, and pressed. The damp material was dissolved in 100 mL of methanol, sodium borohydride (0.6 g) was added in one portion, and after 10 min stirring 0.2 g additional borohydride was added. After 1 h additional stirring, 2.0 g of potassium carbonate was added and stirring was continued for 18 h at room temperature. The material was extracted into chloroform, washed with water, and dried over potassium carbonate. Evaporation of the solvent yielded 0.81 g (93%) of crude material. The product recrystallized from methanol had a melting point of 225–227 °C. TLC (1:1 ethyl acetate–cyclohexane) indicated only one component visualized by an iodine spray. Ir (KBr) 3400 (OH) and 1075 (CO) cm^{-1}; mass spectrum (70 eV) *m/e* (assignment), 397 (M^+), 396 (M − H), 382 (M − CH_3), 204, 150, 136 (typical solanidine fragments) (Budzikiewicz et al., 1964); NMR ($CDCl_3$) δ 0.88 (18-CH_3), 1.01 (19-CH_3 and 21,27-CH_3 doublets), 5.35 (C-6 vinyl).

Other compounds (Sato and Ikekawa, 1961) were synthesized by essentially similar reactions. (22*S*,25*S*)-5α-Solanidan-3β-ol was converted to its C-22 epimer, (22*R*,25*S*)-5α-solanidan-3β-ol, using platinum oxide catalyst in a hydrogen atmosphere (Schreiber et al., 1964). A synthesis (Sato and Ikekawa, 1961) of (22*R*,25*R*)-5α-solanidan-3β-ol failed to produce enough of this compound for experimentation.

Bioassay. The methods used for biological testing were previously described (Brown and Keeler, 1978).

RESULTS AND DISCUSSION

Biological Testing. The results of feeding test compounds are given in Table I along with control hamsters fed jervine or water. Solanidans gave abnormalities in the following order of frequency percentage: Harelip and/or cleft palate (48), cranial bleb (26), microphthalmia (15), exencephaly (10), and cebocephaly (<1). Jervine caused grossly similar malformations at an about similar incidence except that the incidence of cebocephaly was considerably increased. As shown in Table I, compounds having the (22*S*,25*R*) configuration were at least as teratogenic as jervine on a dose–response basis per offspring or litter. The compound having (22*S*,25*S*) configuration caused only an increased resorption rate, and (22*R*,25*S*)-5α-solanidan caused in addition a single abnormal offspring, which may not necessarily be significant.

Conformational Analysis. The usual structure of naturally occurring steroidal indolizidines is (22*R*,25*S*) (Schreiber, 1970). The configuration was established by x-ray analysis (Höhne et al., 1966) and showed the indolizidine conformation was trans. Höhne suggested that (22*S*)-solanidans might have cis indolizidine conformation because of reduced IR absorption in the 2700–2800 cm^{-1} region (Bohlmann, 1958) and failure of the same compounds to undergo mercuric acetate oxidation while (22*R*) epimers react normally. The suggestion by Höhne may, however, be unwarranted for a number of reasons.

Recently the first naturally occurring (22*S*,25*R*)-solanidan ring system was isolated (Pakrashi et al., 1977). The compound was found to have a trans indolizidine conformation and reacted normally with mercuric acetate. It is worth noting that the compound had a 23*S* hydroxyl group (equatorial) which might have some effect on conformation and certainly would change reactivity in mercuric acetate oxidation.

Studies on the 2700–2800 cm^{-1} IR band of indolizidines have shown it is sensitive to substitution for axial hydrogens adjacent and trans to the nitrogen nonbonding electrons (Lüning and Lundin, 1967). The band centered

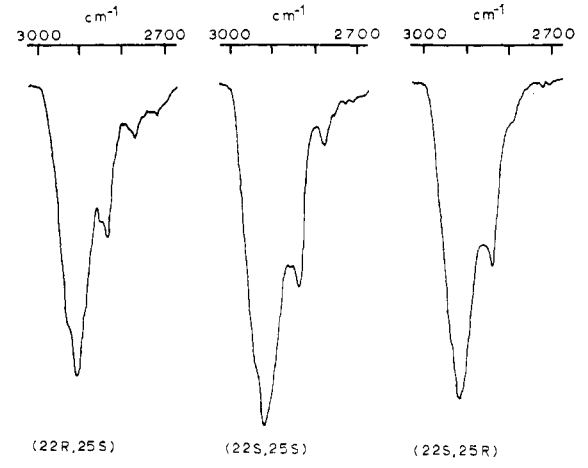

Figure 2. The IR of 5α-solanidan epimers in the C–H stretching region.

at 2885 cm^{-1} was reduced by an unexpectedly large amount in indolizidine-3,3-d_2 (Theobald and Lingard, 1968) and was also concentration dependent. The IR spectra in the 2700–3000 cm^{-1} of three solanidans are shown in Figure 2. The strong absorption centered at 2843 cm^{-1} is not changed appreciably in the three spectra but the smaller band at 2885 cm^{-1}, resolved as a prominent shoulder in the (22*R*,25*S*) epimer, is barely visible in the (22*S*) compounds. Deuteration adjacent to the nitrogen in the indolizidine five-membered ring is known to sharply reduce this absorption (2885 cm^{-1}) as indicated above. If a trans indolizidine conformation is assumed for (22*S*) solanidans, the C-16 hydrogen will not be trans pseudoaxial to the nitrogen nonbonding electrons and a reduced absorption at 2885 cm^{-1} would be expected.

The failure of (22*S*) solanidans to form enamines by mercuric acetate oxidation may also be explained without ruling out a trans conformation of the indolizidine skeleton. Step two in the mechanistic scheme proposed for the reaction involves attack on the tertiary trans axial proton (C-22) (Leonard et al., 1955). The C-22 hydrogen in (22*S*) solanidans is pseudoaxial β (assuming a trans indolizidine) and highly hindered by the axial methyl group C-18. Thus failure of (22*S*) epimers to react with mercuric acetate while (22*R*) solanidan react normally may be due to the steric problems associated with the C-22 hydrogen on the β face of the steroid.

Free energy considerations also favor a trans conformation in the indolizidine skeleton of solanidans. The $\Delta G°$ of cis ⇄ trans isomerization of indolizidine has been estimated to be −2.4 kcal/mol (Aaron and Ferguson, 1968). It is reasonable to assume the energy required to invert solanidans would be even larger because of the added ring strain caused by the cis fused D,E rings. Table II gives the conformation of various groups in the E and F rings of solanidans assuming a trans indolizidine skeleton. Note that (22*S*) epimers give rise to a pseudoaxial methyl group (C-21) and the C-27 methyl is axial β for the (22*R*,25*R*) configuration and axial α for the (22*S*,25*S*) structure. Nevertheless, there do not appear to be interactions sufficient to cause inversion of the indolizidine skeleton in any of the C-22, C-25 epimeric solanidans.

An additional point regarding the conformation of the indolizidine skeleton of solanidans may be important to their teratogenic activity. The very small $\Delta G°$ of cis ⇄ trans isomerization of hydrindane (0.3 kcal/mol) was attributed to ring strain in the trans compound which is relieved in the cis configuration (Allinger and Coke, 1960). The relatively large $\Delta G°$ for the same transformation in

Table II. Conformational Analysis of the E and F Rings of Solanidan

Configuration		Nitrogen nonbonding	C-21[a]	C-27
C-22	C-25	electrons	methyl group	methyl group
R	S	β	e	e
R	R	β	e	a(β)
S	S	α	a	a(α)
S	R	α	a	e

[a] This methyl is pseudoaxial or pseudoequatorial.

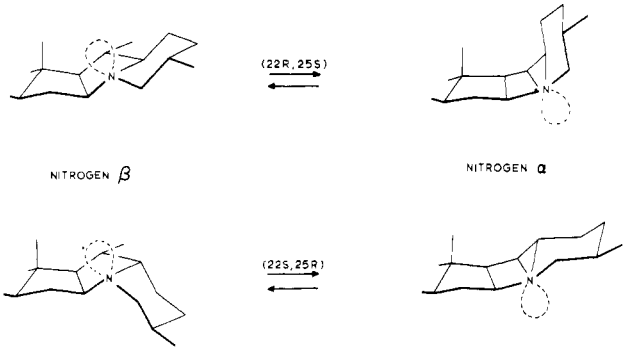

Figure 3. Solanidan conformers produced by nitrogen inversion.

indolizidine was attributed to "flattening" of the bridgehead nitrogen thus relieving strain in the trans conformer (Crabb and Newton, 1970). A similar effect has been noted in free-energy studies of a number of heterocyclic systems. It is important to note that a deformation of the tetrahedral (sp^3) character of the nitrogen will tend to add p character to the nonbonding electrons and thus add negative charge to the back side of the nitrogen. This negative character has previously been shown to be important to teratogenic activity when placed α to the D ring in both jervine (Brown and Keeler, 1978) and spirosolane (Keeler et al., 1976a) related compounds.

Structure–Activity. The dramatic differences in teratogenic potency of solanidan epimers supports the notion that a basic nitrogen, accessible to the α steroid face, is required for activity. (22*R*,25*S*)-5α-Solanidan-3β-ol, conformationally unfavorable to having a nitrogen α (Table II), gave no significant incidence of abnormalities. The (22*S*,25*S*) epimer does not give a significant incidence of abnormalities even though the conformation predicts nitrogen α. This compound may not be teratogenic because the nitrogen nonbonding electrons are shielded from active sites by the 1,3-diaxial interaction with the methyl group in ring F (C-27). The (22*R*,25*R*) epimer is predicted to be inactive on conformational grounds, but has not been tested. (22*S*,25*R*)-Solanidans, conformationally most favorable to placing a nitrogen base accessible to the α face of the steroid, were relatively potent teratogens.

These findings relate to the hypothesis (Renwick, 1972) that potatoes, which contain solanidine, can be teratogenic. Solanidine (**5**, Figure 1) with (22*R*,25*S*) configuration is not likely to be nitrogen α (see also Figure 3) and thus would not be predicted to be highly active. Nevertheless, the placement of the nitrogen nonbonding electrons in these molecules is conformational as shown in Figure 3 and the previous section discussed a possibility of increased negative character to the back side of the nitrogen. We may also conclude that the solanidine nitrogen could invert (Figure 3) and the inverted conformer would be active if other conformational factors did not interfere. Recent findings with chick embryo assay (Jelínek et al., 1976; Mun et al., 1975) and hamsters (Keeler et al., 1976b) support

the proposal that solanidine and its glycosides can cause birth defects when fed during embryonic differentiation, although the risk of such defects may be quite small. The data (Table I) apparently support this proposal showing that (22*R*,25*S*)-5α-solanidan-3β-ol caused relatively high percentages of fetal resorption in as much as resorption and malformation rates are generally closely related. The single abnormal offspring, although not a significant incidence, was a characteristic defect of teratogenic steroids. It is also apparent from the widely different structures of the highly teratogenic compounds, jervine and (22*S*,25*R*)-5α-solanidan-3β-ol, that structural requirements for activity (aside from an unhindered basic nitrogen accessible to the α steroid face) are minimal and that a conventional steroid skeleton fitting this requirement can be highly teratogenic.

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LITERATURE CITED

Aaron, H. S., Ferguson, C. P., *Tetrahedron Lett.*, 6191 (1968).
Allinger, N. L., Coke, J. L., *J. Am. Chem. Soc.* **82**, 2553 (1960).
Brown, D., Keeler, R. F., *J. Agric. Food Chem.*, previous two papers in this issue (1978).
Bohlmann, F., *Chem. Ber.* **91**, 2157 (1958).
Budzikiewicz, H., Djerassi, C., Williams, D. H., "Structure Elucidation of Natural Products by Mass Spectrometry", Vol. 2, Holden-Day, San Francisco, Calif., 1964.
Crabb, T. A., Newton, R. F., *Tetrahedron Lett.*, 1551 (1970).
Höhne, E., Schreiber, K., Ripperger, H., Worch, H. H., *Tetrahedron* **22**, 673 (1966).
Jelínek, R., Kyzlink, V., Blattný, C., Jr., *Teratology* **14**, 335 (1976).
Keeler, R. F., Young, S., Brown, D., *Res. Commun. Chem. Pathol. Pharmacol.* **13**, 723 (1976a).
Keeler, R. F., Brown, D., Douglas, D. T., Stallknecht, G. F., Young, S., *Bull. Environ. Contam. Toxicol.* **15**, 522 (1976b).
Kuč, J., *Recent Adv. Phytochem.* **9**, 139 (1975).
Kusano, G., Aimi, N., Sato, Y., *J. Org. Chem.* **35**, 2624 (1970).
Leonard, N. J., Hay, A. S., Fullmer, R. W., Gash, V. W., *J. Am. Chem. Soc.* **77**, 439 (1955).
Lüning, B., Lundin, C., *Acta Chem. Scand.* **21**, 2136 (1967).
Mun, A. M., Barden, E. S., Wilson, J. M., Hogan, J. M., *Teratology* **11**, 73 (1975).
Pakrashi, S. C., Chakravarty, A. K., Ali, E., *Tetrahedron Lett.*, 645 (1977).
Renwick, J. H., *Brit. J. Prev. Soc. Med.* **26**, 67 (1972).
Sato, Y., Ikekawa, N., *J. Org. Chem.* **26**, 1945 (1961).
Schreiber, K., Horstmann, C., Adam, G., *Chem. Ber.* **97**, 2368 (1964).
Schreiber, K., *Pure Appl. Chem.* **21**, 131 (1970).
Seifulla, Kh. I, Ryzhova, K. E., *Mater. Vses. Konf. Issled. Lek. Rast. Perspekt. Ikh Ispol'z Proizvod. Lek. Prep.* **1970**, 160 (1972); *Chem. Abstr.* **83**, 22383t (1975).
Theobald, A. E., Lingard, R. G., *Spectrochim Acta, Part A* **24**, 1245 (1968).

Received for review September 6, 1977. Accepted December 23, 1977. Presented in part at the 173rd National Meeting of the American Chemical Society, Division of Medicinal Chemistry, New Orleans, La., Mar 1977. Support was provided by interagency agreement between the Agricultural Research Service, U.S. Department of Agriculture 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.

Adsorption and Desorption of Parathion by Attapulgite as Affected by the Mineral Structure

Zev Gerstl* and Bruno Yaron

Adsorption of parathion by pretreated attapulgites was studied in both organic and aqueous media. In organic media the presence of hygroscopic moisture resulted in competition between parathion and water so that an increase in the clay's moisture content reduced parathion adsorption. Heating the clays above 250 °C resulted in structural changes that cause a decrease in parathion adsorption. Adsorption of parathion in aqueous solution was found to be inversely related to the release of parathion from highly loaded clays under equilibrium conditions. Saturating clays with an organocation altered their properties favoring increased adsorption and decreased desorption. The results indicate that pretreatment of the clay might affect both the rate and amount of release to the external environment.

For insoluble or slightly soluble pesticides, dust formulations are commonly used to achieve uniform application to the soil surface; the desired compound is added in combination with a solid diluent (Polon, 1973). Attapulgite is one of the clays commonly used. This mineral is a fibrous clay which differs from the more common layer silicates (montmorillonite, kaolinite) in that it does not consist of discrete platelets, but rather has a rigid three-dimensional structure with microchannels (3.5 × 6 Å) running the length of the structure (Bradley, 1940). It contains three forms of water: zeolitic water, which is lost below 220 °C; crystal water coordinated to magnesium ions in the crystal and lost upon being heated at 225–350 °C; and hydroxyl water of the silicate unit, lost above 400 °C (Haden and Schwint, 1967). Heating the clay to various temperatures, therefore, will have a profound influence not only on the clay's water content but upon its structure as well.

Despite the wide use of attapulgite as a carrier, only little attention has been paid to its interactions with pesticides. Early studies by Fowkes et al. (1960), Polon and Sawyer (1962), and Rosenfield and Van Valkenburg (1965) reported the behavior of several organochlorinated and

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