unresponsive to corticosteroid-like agents (15). The results of the present study do not conflict with this theory and do establish that the anti-inflammatory activity of cryogenine probably is not due to a mercaptopurine-like immunosuppressive capacity.

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Structure–Side-Effect Sorting of Drugs II: Skin Sensitization

ERIC J. LIEN▲ and GEDY A. GUDAUSKAS*

Abstract □ A computerized sorting program was used to sort out 120 drugs that have been reported to cause skin sensitization from a data bank of 540 clinically useful drugs. Based on an analysis of functional groups, a number of unique common denominators were found for skin sensitization due to covalent bond formation with body proteins. These include potential epoxide-forming groups such as an electron-deficient benzene ring, a vinyl group, hydroxylamine and *N*-oxide precursors, drugs capable of forming a benzylic radical, β-lactam derivatives, and α,β-unsaturated ketones. By using multiple-regression analysis, the quantitative structure–activity relationship for skin-sensitizing properties of 3-alkyl catechols and 2,4-dinitrobenzene derivatives are also reported.

Keyphrases □ Side effects, computer sorting of 540 drugs—skin sensitization related to structure □ Computer sorting of side effects of 540 drugs—skin sensitization related to structure □ Structure–activity relationships—features related to skin sensitization effects, result of computer sorting of 540 drugs □ Skin sensitization effects—related to structural features, result of computer sorting of 540 drugs □ Drug sorting by side effects—structure–activity relationships

As a continuation and extension of an effort to sort and correlate side effects of drugs with their chemical structures (1), attention was focused on the problem of skin sensitization. Numerous drugs are known to cause dermatitis, urticaria, eczema, photosensitization, or other signs of skin sensitization following either systemic or topical applications. The main purpose of this study was to utilize a computerized sorting program to list as many as possible of the drugs known to cause various types of skin sensitization and to find out the common structural features. Attempts were made to categorize these drugs on the basis of functional group analysis and the types of possible chemical reactions with proteins. It is hoped that the findings will not only suggest working hypotheses for skin sensitization by various drugs but will also provide some rational chemical basis of skin sensitization. Quantitative structure–activity analysis was applied to a series of catechols and dinitrobenzene derivatives where quantitative data on series of compounds were available.

METHOD

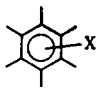
The computerized sorting program was described previously (1). The following descriptive terms of signs were sorted out from the data bank: skin sensitization, lesions, necrosis, rashes, eruptions, reactions, irritations, opacities, dermatitis, photosensitivity, and urticaria. The results are given in Tables I and II.

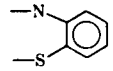
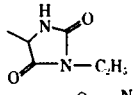
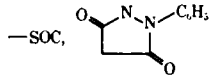
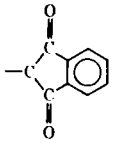
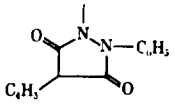
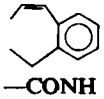
The method of least squares was used to derive the equations correlating the physicochemical parameters with the delayed contact skin-sensitizing activity. The biological data and the physicochemical constants used are assembled in Table III.

RESULTS AND DISCUSSION

Skin-Sensitizing Drugs—A total of 120 drugs or drug groups from the data bank of 540 drugs have been reported to cause various types of skin sensitization (2–8). In analyzing the common structural features, many of these drugs were found to contain a benzene ring

Table I—Skin-Sensitizing Drugs Possessing Aromatic Ring Bearing Electron-Withdrawing Groups



Drug	X	Drug	X
Benzonatate	—$COOCH_2$—	Chloroquine	—Cl, —N=C
Benztropine	—$OCHC_6H_5$	Chlorphenesin	—Cl, —OCH_2—
Butaperazine	—CO′ —S, N—C_6H_5	Clofibrate	—Cl, —OC
Brompheniramine	—Br	Cyproheptadine	—C=C, —C=C
Carbamazepine	—$NCONH_2$, C=C—C_6H_5	Diethylpropion	—C—C(=O)
Carphenazine	—C(=O)—C_2H_5	Diphemanil	—C(—C_6H_5)=
Chlorphentermine	—Cl	Diphenylhydantoin	—C(—C_6H_5)—C(=O)
Chlorprothixene	—Cl	Dipyrone	—N—C(=O)
Chlordiazepoxide	—Cl, —N=; —C(—C_6H_5)=N→O	Doxepin	—C=O, —O—
Chlormezanone	—Cl, —CSO_2	Ethopropazine	
Glycopyrrolate	—C(OH)(—COO)	Ethotoin	
Indomethacin		Sulfinpyrazone	
Methadone	—C(—C_6H_5)—C(=O)	Suramin	—CO, —SO_2Na
Phenindione		Mesoridazine	—SO—, —S—, —NC_6H_5
Phenothiazines	—S, —N—C_6H_5	Thiothixene	—C=, —S
Phenylbutazone		Triflupromazine	—CF_3, —S, —NC_6H_5
Propranolol			
Protriptyline			
Procarbazine	—CONH		

or other aromatic ring systems bearing electron-withdrawing groups (Table I). The electron-withdrawing groups may stabilize the epoxide formed by drug-metabolizing enzymes. It has been shown (9) that drug-metabolizing enzymes in the liver cause the formation of active epoxides from bromobenzene, *o*-dibromobenzene, *m*-dibromobenzene, and naphthalene (10). These epoxides may then alkylate body protein or cause tissue damage in liver, kidney, bone marrow, *etc.* (11, 12). Other compounds such as various isomers of octene (13), styrene (14), and chlordane (15) also have been shown to form epoxides *in vivo*. The carbon–carbon double bond in diethylstilbestrol, carbamazepine, and novobiocin may be responsible for the skin sensitization *via* epoxide formation.

The second group of drugs that has been reported to cause skin sensitization contain either a primary aromatic amine moiety with the *para*-position blocked or a tertiary aliphatic amino group (Table II). Compounds with the structure of X—C_6H_5—NH_2 form the highly reactive and toxic hydroxyamino and nitroso compounds (16–18), which may then react with proteins to form antigens. Schwarz and Speck (19) demonstrated that hydroxyamino derivatives of sulfanilamide play a role in photosensitivity. They were able to sensitize guinea pigs with these oxidation products of sulfanilamide and then to provoke a reaction by exposure to sulfanilamide and light and vice versa.

In contrast to the sulfanilamides, when patients known to be photoallergic to chlorothiazides were skin tested to the oxidation products 4-amino-6-chlorobenzenedisulfonamide and 4-hydroxyamino-6-chlorobenzenedisulfonamide, no positive reactions were elicited in patch and intracutaneous tests and in irradiated patch and intracutaneous tests with these compounds (20). Therefore, the exact mechanism by which drugs like chlorothiazides induce skin sensitization remains to be investigated. Since the positions *meta* and *para* to the chloro atom are blocked by the thiazide ring, the formation of an epoxide is not likely.

The nitro group in chloramphenicol is also known to undergo reduction to give an amino group (21). This minor metabolic path-

Table II—Skin-Sensitizing Drugs Possessing Amino Group

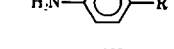
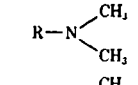
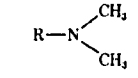
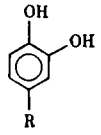
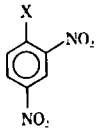
Drug	Functional Group
Sulfonamides	
Phenothiazines	
Imipramine	

Table III—Biological Data and Physicochemical Constants of Catechols and Dinitrobenzenes Causing Delayed Contact Sensitivity in Guinea Pigs



3-Alkyl Catechols

log $1/C$[a]	log P[b] (Octanol–Water)	R
4.89	0.88	H
5.35	1.38	CH_3
5.77	2.38	n-C_3H_7
6.01	3.38	n-C_5H_{11}
6.72	4.88	n-C_8H_{17}
7.37	6.38	n-$C_{11}H_{23}$
6.89	7.38	n-$C_{12}H_{27}$
7.27	8.38	n-$C_{15}H_{31}$
7.42	9.38	n-$C_{17}H_{35}$
7.46	10.38	n-$C_{19}H_{39}$



2,4-Dinitrobenzene Derivatives

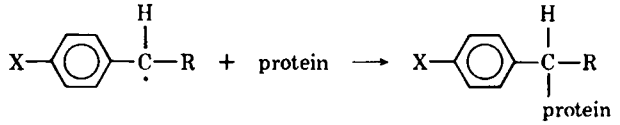
log $1/C$[c]	log P[d] (Isooctane –Water)	log P[e] (Octanol–Water)	V_x[f]	log R[g]	X
8.91	0.66	2.58	42.7	1.78	—SCN
8.72	1.63	2.41	37.6	1.00	—I
8.62	1.15	2.24	27.6	0.86	—Br
8.32	1.26	2.08	21.3	0.97	—Cl
8.17	0.51	1.50	8.54	0.15	—F
7.83	−0.48	—	—	—	—SO_2Cl
7.32	−2.30	−2.89	—	1.89	—SO_3H

[a] C = the geometric mean dose (moles) for threshold skin reaction 4 weeks after the animals received the last sensitizing injection; taken from Table I of *Reference 30*. [b] Based upon the experimental value of 0.88 for pyrocatechol and use of π = 0.50 for each CH_3 or CH_2 unit (36). [c] C = the geometric mean dose (moles) for threshold skin reaction (homologous) 47 days after sensitization; taken from Table I of *Reference 33*. [d] Experimental values from *Reference 33*. [e] Calculated by taking advantage of the additive–constitutive nature of the Hansch π constant. The following π values are used: 1,3-dinitrophenyl = 1.49; SCN = 1.01; I = 0.92; Br = 0.75; Cl = 0.59; F = 0.01; and SO_3H = −4.38; taken from *Reference 35*, phenoxyacetic acid system, and *Reference 36*. [f] Volume in Å^3 from *Reference 33*. [g] R = reactivity coefficient with bovine serum albumin (BSA), R = (hapten)/(DNP–BSA) − 1, where DNP = dinitrophenol. For the experimental procedure, see *Reference 33*.

way may be responsible for the skin sensitization. Another possible way of forming a covalent bond between chloramphenicol and a protein would be *via* a benzylic free radical, as suggested by Hansch and Kerley (22) (*vide infra*).

The third group of drugs contains substituents which may stabilize a benzylic radical (22). This group includes drugs such as chloramphenicol and quinine (Scheme I).

It is also known that various aliphatic tertiary amino compounds form *N*-oxides in many biological systems (Scheme II) (16, 24, 26). Since these *N*-oxides can be isolated fairly easily, they probably are not as reactive as *N*-nitroso or *N*-hydroxyamine derivatives. Nevertheless, it has been reported that in several cases *N*-oxides are more active than their corresponding tertiary amines (27).



Scheme I

Table IV—Equations Correlating Skin-Sensitizing Properties with Physicochemical Constants

	n[a]	r[b]	s[c]	Eq.
3-Alkyl Catechols				
$\log 1/C = 0.262 \log P + 5.080$	10	0.946	0.326	1
$\log 1/C = -0.030 (\log P)^2 + 0.591 \log P + 4.488$	10	0.983	0.169	2
$\log P_o = 17.96 (14.44 - 31.81)$[d]				
2,4-Dinitrobenzene Derivatives				
$\log 1/C = 0.146 \log P_{(\text{isooctane-water})} + 8.396$	5	0.233	0.338	3
$\log 1/C = 0.448 \log R + 8.121$	5	0.864	0.175	4
$\log 1/C = 0.257 \log P + 8.004$	6	0.946	0.206	5
$\log 1/C = 0.022 V_x + 7.951$	5	0.975	0.077	6

[a] n = number of data points (compounds used in the regression analysis). [b] r = correlation coefficient. [c] s = standard deviation. [d] 95% confidence interval.

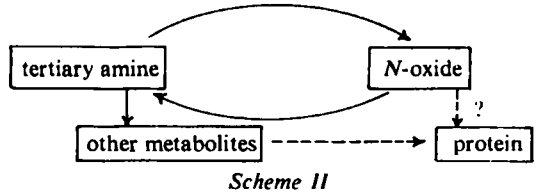
Some *N*-oxides also have been postulated as inducers of spontaneous cancer. These *N*-oxides may also be important intermediates in drug metabolism (27).

The fourth group of drugs causing skin sensitization and/or anaphylactic shock contains drugs with a β-lactam moiety. The four-membered β-lactam ring may open up and form a new peptide linkage with body protein (Scheme III) (28). All of the penicillins and cephalosporins belong to this group.

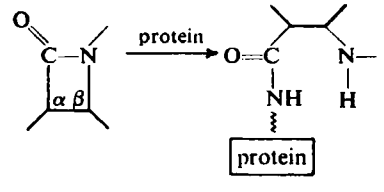
The fifth group of drugs which can form covalent bonds with proteins and cause skin sensitization includes those possessing an α,β-unsaturated ketone function. The examples found from the data bank are cortisone, digoxin, griseofulvin, and spironolactone. These drugs can conceivably undergo nucleophilic attack by any nucleophiles ($\ddot{N}u$) such as —SH, —O^-, and —$\ddot{N}H_2$ (Scheme IV) (29).

The sixth group of skin-sensitizing drugs contains polyiodinated benzene derivatives, *e.g.*, ipodate, iothalamic acid, and diiodohydroxyquin. Polyiodinated benzene derivatives are readily deiodinated *in vivo* (30, 31). Although the exact mechanism is not known, this process possibly may go through a free radical intermediate. The iodine radical, the phenyl radical, or the iodide ion produced may act as a hapten and react with protein.

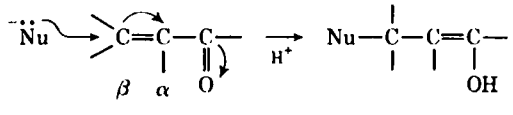
Miscellaneous Compounds—Drugs like kanamycin and paromomycin contain primary amino groups and sugar moieties. It



Scheme II



Scheme III



Scheme IV

does not appear likely that these molecules would form covalent bonds with body protein. Whether the skin sensitization caused by these drugs is due to the trace amount of fermentation by-products by *Streptomyces* or not remains to be studied.

Other drugs like phenacemide, phenacetin, phenelzine, and barbiturates are also known to cause sensitization. No well-established biotransformations or chemical reactions are available to explain the coupling between these compounds and proteins. It is possible that small amounts of reactive metabolites may be responsible for the skin sensitization reported. Identification of these metabolites will be essential for the establishment of the mechanism.

Quantitative Structure–Activity Relationship—The equations correlating the skin-sensitizing properties with the physicochemical constants are summarized in Table IV. The biological data are from Baer *et al.* (32) and Godfrey and Baer (33). It has been suggested that the mechanism of sensitization by compounds of the catechol type results from conversion to a quinone, which then spontaneously couples to protein (34). Equations 1 and 2 clearly indicate that the major variation in the skin-sensitization activity of the 3-alkyl catechol is primarily due to the lipophilic character (log P) or the length of the side chain, as indicated by the log P from octanol–water. However, because all of the substituents are straight-chain alkyl groups, there is a strong correlation between the log P and the chain length. A better selection of substituents with different steric and lipophilic character may delineate which parameter is more important in contributing to the skin sensitization. The $(\log P)^2$ is significant at the 99 percentile level as indicated by an F-test ($F_{1,7} = 15.05$, $F_{1,7,99} = 12.2$). About 97% of the variance ($r^2 = 0.97$) in the data can be accounted for by the parabolic equation of log P (Eq. 2). The optimum lipophilic character for maximum sensitization is defined by log P_0 (37, 38). The log P_0 of 17.96 from Eq. 2 is considerably higher than that of pentadecylcatechol (log $P = 8.38$), the active principle of poison ivy. The fairly wide 95% confidence interval of log P_0 is primarily due to the fact that the highest log P in the series is only 10.38. Nevertheless, the statistical analysis points to the deviation from linearity.

For the 2,4-dinitrobenzene derivatives, Eqs. 3–6 are obtained. Among the parameters examined, the molar volume of the substituent (V_x) gives a slightly better correlation than log P (octanol–water) or the reactivity coefficient (R) with bovine serum albumin. Pure hydrocarbon is known to yield less satisfactory correlation with biological activity than the solvents that are capable of forming hydrogen bonds. This becomes very evident when one compares Eqs. 3 and 5. The log P of 2,4-dinitrobenzenesulfonyl chloride in octanol–water is not available because it reacts with alcohols, and that of 2,4-dinitrobenzenesulfonic acid in isooctane–water is not available because of the very low solubility in the hydrocarbon. As a consequence, six data points are included in Eq. 5, but only five data points are in Eqs. 3 and 6. It is difficult to assess whether Eq. 6 is really better than Eq. 5. Intercorrelation between the volume of the substituent (V_x) and log P_{octanol} is also apparent ($r = 0.98$). The analysis is in agreement with the original authors' conclusion (33) regarding the importance of the steric nature of the substituent. The results also show that if a proper solvent system is chosen, one can get practically the same correlation by using log P instead of the steric parameter. A better selection of substituents with no interdependence between log P and V_x will be necessary to evaluate the contribution of these two parameters to skin sensitization.

Since the phototoxic reaction does not involve the conjugation of hapten with protein (39, 40), it is not included in this study. For the general discussion of allergic drug reactions, a recent article by MacFarlane (41) is available.

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