did not change significantly from control values; presumably, hepatic blood flow behaves similarly. The extraction ratio for lidocaine in the pregnant sheep was 0.57 using Eq. 5. Similar assumptions and calculations for the nonpregnant sheep [Q = 55 ml/min/kg (14)] yielded an extraction ratio of 0.69. These values are similar to the extraction ratio (~0.68) reported for lidocaine in humans (17).

The results of these studies provide evidence for subtle dose-related lidocaine kinetics in pregnant sheep. Further studies on lidocaine binding in ovine plasma and on cardiac output and blood flow distribution during the initial lidocaine distributive phase in sheep are necessary to substantiate the reasons for the apparent nonlinearity of lidocaine disposition.

REFERENCES

(1) G. T. Tucker and R. A. Boas, *Anesthesiology*, **34**, 538 (1971).

(2) G. T. Tucker and L. E. Mather, *Br. J. Anaesth. Suppl.*, **47**, 213 (1975).

(3) L. E. Mather and G. T. Tucker, *J. Pharm. Sci.*, **63**, 306 (1974).

(4) C. M. Metzler, G. L. Elfring, and A. J. McEwen, *Biometrics*, **30**, 562 (1974).

(5) K. S. Albert, A. J. Sedman, and J. G. Wagner, *J. Pharmacokinet. Biopharm.*, **2**, 381 (1974).

(6) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975, pp. 48, 68, 177.

(7) J. G. Wagner, *J. Pharmacokinet. Biopharm.*, **1**, 363 (1973).

(8) J. J. Bonica, P. U. Berges, and K. Marokawa, *Anesthesiology*, **33**, 619 (1970).

(9) J. Thomsen, W. Terry, R. Stenlund, S. Queremit, and G. Rowe, *Arch. Int. Pharmacodyn. Ther.*, **194**, 83 (1971).

(10) M. Rowland, P. D. Thomson, A. Guichard, and K. L. Melmon, *Ann. N.Y. Acad. Sci.*, **179**, 383 (1971).

(11) G. T. Tucker, R. N. Boyes, P. O. Bridenbaugh, and D. C. Moore, *Anesthesiology*, **33**, 287 (1970).

(12) P. D. Thomson, K. L. Melmon, J. A. Richardson, K. Cohn, W. Steinbrunn, R. Cudihee, and M. Rowland, *Ann. Intern. Med.*, **78**, 499 (1973).

(13) K. A. Collinsworth, S. M. Kalman, and D. C. Harrison, *Circulation*, **50**, 1217 (1974).

(14) M. L. Katz and E. N. Bergman, *Am. J. Physiol.*, **216**, 946 (1969).

(15) G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 377 (1975).

(16) H. O. Morishima, M. Finster, A. Fukunaga, H. Pedersen, R. N. Boyes, and B. G. Covino, "Abstracts of Scientific Papers," American Society of Anesthesiologists Annual Meeting, Chicago, Ill., 1975, pp. 85, 86.

(17) G. T. Tucker, L. Wiklund, A. Berlin-Wahlen, and L. E. Mather, *J. Pharmacokinet. Biopharm.*, **5**, 111 (1977).

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False-Positive Alkaloid Reactions

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Received February 15, 1979, from the *Faculty of Pharmacy, Alexandria University, Alexandria, Egypt.* Accepted for publication July 20, 1979.

Abstract □ A variety of nonnitrogenous oxygenated compounds gave false-positive alkaloid reactions with Dragendorff's spray reagent. These compounds reacted positively if the oxygen function and the β-carbon bonded to the oxygen had high electron density. Thus, aldehydes, ketones, lactones, ethers, esters, epoxides, and peroxides with an ethylene bond or free alkyl groups at the β-carbon gave a positive reaction, provided that the availability of electrons at the oxygen and the β-carbon was not altered by electron withdrawal or hydrogen bonding. Carbonyl, ether, and ethylene functions were shown by IR evidence to be involved in coupling. Nitrogen-free, alkaloid-like acetone artifacts were obtained by interaction with fixed alkali and with acids. These compounds were postulated to be α,β-unsaturated aldol condensation products of acetone. Interaction with ammonia in addition yielded nitrogenous alkaloid-like artifacts.

Keyphrases □ Alkaloids—interference by nonnitrogenous alkaloid-like compounds, structural requirements for false-positive reaction □ Acetone artifacts—nonnitrogenous, alkaloid-like compounds, interference with alkaloid detection, structural requirements for false-positive reaction □ Dragendorff's reagent—false-positive alkaloid reaction with nonnitrogenous oxygenated compounds

Of the numerous reagents described for the detection of alkaloids (1), only a few have reliable sensitivity (2). All of these reagents suffer from nonspecificity. Many nitrogenous and nonnitrogenous plant constituents react with several of these reagents similarly to alkaloids (3–8). Some nonnitrogenous compounds react similarly to alkaloids in giving crystalline salts with acids (8).

BACKGROUND

TLC is the most versatile technique for alkaloid detection, separation, monitoring, identification, and quantitation. Dragendorff's spray, in its different modifications, is usually used for visualization of alkaloidal spots on paper and thin-layer chromatograms and in field tests with alkaloid test paper (9–11). Many reports have discussed its sensitivity and specificity (2), false reactions (3, 12), and modifications (13–20). However, many nonnitrogenous plant constituents react with this reagent in a manner typical of alkaloids (3, 12, 21). Such compounds may create difficulty, especially during alkaloid screening without sufficient partition purification steps (4). Complete elimination of these constituents cannot be accomplished through the one partition purification step required in many reported screening procedures (22–26). Moreover, significant amounts of such nonalkaloidal constituents may be detected even after further partition purification (12).

Farnsworth *et al.* (3) determined that any nonnitrogenous compound having conjugated carbonyl or lactone functions would react in a manner typical of alkaloids. The range of compounds that produce a false-positive reaction is greater than is generally realized (27), and compounds such as the common plant sterols and triterpenes are readily detected by Dragendorff's spray.

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Table I—Compounds Tested in the Present Study that Reacted Positively with Dragendorff's Spray Reagent

Column A: Compounds with Conjugated Carbonyl or Lactone Functions		Column B: Compounds Lacking any Conjugated Carbonyl or Lactone Function	
Compound	Reaction[a]	Compound	Reaction[a]
With carbonyl or lactone as only reactive function		Nonconjugated aldehydes and ketones	
Conjugated aldehydes and ketones		Isobutyraldehyde	+++
Acrolein	+++	Menthone	++
Crotonaldehyde	+++	Camphor	++
Furfural	+++	Acetonylacetone	+
Cinnamic aldehyde	+++	Nonconjugated lactones	
Citral	++	Picrotoxin (picrotoxinin)	++
Carvone	+++	Arctiin	+++
3-Hydroxybenzaldehyde	+(±)	Isolipidiol	+
Acetophenone	+++	*Judeicin*	+
Benzophenone	++	α-β-Unsaturated alcohols (their ethers and esters)	
Chalcone	+++	Geraniol	+(±)
Benzil	++	Geranyl acetate	+
Progesterone	+	Linalyl acetate	+
Testosterone	+	Phenols, phenolic ethers, and esters	
Cortisone	++	Eugenol	+++
Conjugated lactones		Acetyleugenol	+++
Coumarin	+++	Anethole	+
Umbelliferone	+	Guaiacol	+(±)
Digitoxin	+++	Thymol	+(±)
Digoxin	+++	Acetylated thymol	+
Strophanthin	++	α,β-Unsaturated (or aromatic) acids and their esters	
Ouabain	+	Cinnamic acid	+(±)
Acovenosides[b]	+++	Ethyl cinnamate	+
Lactone *S.a.*[c]	+	Phenyl salicylate	+
With additional reactive ether or ester functions		Methyl salicylate	+
Piperonal	+++	Veratric acid	+++
Veratraldehyde	+++	Alcohols (their ethers and esters) with branching at β-carbon	
Anisaldehyde	++	Primary isobutanol	+
Vanillin	+(±)	Primary isobutyl acetate	+++
Acetylated vanillin	++	Isobutyl epoxide	+++
3-Butoxybenzaldehyde	++	Isoamyl alcohol	+
Khellin	+++	Isoamyl acetate	+++
Visnagin	+++	Isoamyl ether	++
Methoxsalen (xanthotoxin)	+++	Menthyl acetate	++
Bergapten	+++	Bornyl acetate	+
Imperatorin	+++	Isobornyl acetate	+
Santonin	+++	Acetylated β-amyrin	++
Xanthumin	++	Miscellaneous compounds	
Rotenone	(+++)[d]	Cineole	++
Total withanolides[e]	+++	*Ascaridole*	+++
Withaferin A	+++	Digitonin	++
A C_{28} steroid lactone from *Withania somnifera*[f]	(+)[f]	Diosgenin	+++
Acetylated rutin	+++	2,3-Dihydro-4-pyrone	+
Acetylated quercitin	++	Ethyl-2-cyclohexanone carboxylate	+++
Acetylated kaempferol glucoside	++	Cannabinoids[g]	+
Acetylated hyperoside	+		
Acetylated quercitin glucuronide	+(±)		
Acetylated quercitin pentoside	+		
Acetylated chrysophanic acid	+		
Acetylated sennidins A + B	+		
β-Glycyrrhetinic acid	+(±)		

[a] Key: +, faint reaction; ++, typical reaction; and +++, immediate typical reaction. [b] The total -olide fraction of *Acokanthera spectabilis*, isolated by a reported method (29), showed at least four Dragendorff-positive components on TLC. [c] A conjugated ketolactone isolated from *Senecio aegyptius* (38). [d] Some compounds that gave a typical orange-red color with the fresh reagent gave a typical bluish color with old reagent (*e.g.*, cortisone and rotenone). [e] The lactonic fraction of *Withania somnifera*, freed from any contaminating alkaloids, showed at least six Dragendorff-positive spots. [f] Isolated from the roots and reported to give a positive reaction with Dragendorff's reagent. [g] Extracted from *Cannabis* and chromatographed by reported methods (33, 34).

Farnsworth *et al.* (3) gave convincing explanations for certain confusing cases (7, 8) as well as certain claims about the detection and isolation of alkaloids (28) that were denied in a subsequent report (29). Nevertheless, the conclusion of these investigators did not apply to 19 of the 29 compounds listed as positively reacting miscellaneous compounds (Ref. 3, Table II, column B). Moreover, it gave no persuasive answer to observations by the present author; phytochemical screening of *Mentha microphylla* and *Lavandula dentata* of Saudi Arabia revealed two constituents in the former and one in the latter species that gave a positive reaction with Dragendorff's spray, and these compounds were identified as menthone, carvone, and camphor (30, 31). Arctiin, a nonconjugated lactonic lignan glycoside, also gave[1] a positive reaction with Dragendorff's reagent as well as with other alkaloid precipitants. The spray reagent was used for visualization of cineole and ascaridole on TLC. Menthone, camphor, cineole, ascaridole, and arctiin lack the minimal structural features determined by Farnsworth *et al.* (3).

This ambiguity prompted the present study to determine the minimal structural features for a false-positive alkaloid reaction with emphasis on the reaction with Dragendorff's spray.

EXPERIMENTAL

Test Compounds—The compounds were obtained from commercial sources. Some natural products were provided by investigators or separated by TLC from plant materials, crude drugs, or preparations. Localization of these compounds was achieved through reliance on reported R_f values in specified TLC systems and the use of specific chromogenic reagents (32–35), and no quantitation was attempted.

Acetylated Compounds—Acetylation of the test compounds was effected by pyridine–acetyl chloride or acetic anhydride–sulfuric acid. Chloroform extracts of the acetylated compounds were washed thoroughly with 2% HCl and water or with 2% $NaHCO_3$ and water, respectively.

[1] Dr. A. A. Omar, Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt, personal communication.

Table II—Compounds Reported by Farnsworth *et al.* (3) to React Positively with Dragendorff's Spray Reagent

Column A: Compounds with Conjugated Carbonyl or Lactone Structure		Column B: Compounds Lacking any Conjugated Carbonyl or Lactone Function	
Compound	Reaction[a]	Compound	Reaction[a]
Ten 4-methoxy-α-pyrones	++++	Eugenol	++++
Four coumarins	++++	Hydroxycitronellol	++++
Four furanocoumarins	++++	Menthyl salicylate	++++
Three γ-pyrones	++++	Phenyl salicylate	+++
Khellin	++++	Salicylic acid	++
Chalcone	++++	Benzyl acetate	++
β-Methoxychalcone	++++	Camphor	++
cis-1,4-Diphenyl-2-butene-1,4-dione	++++	Cineole (eucalyptol)	++
trans-1,4-Diphenyl-2-butene-1,4-dione	++++	Methyl salicylate	++
Acrolein	++++	Aspirin	+
Amyl cinnamate	++++	Anethole	+
Cinnamaldehyde	++++	Cinnamic acid	+
Digitoxin	++++	Geraniol	+
Menadione	++++	Guaiacol	+
Ouabain	++++	Hydroquinone	+
Triketohydrindene hydrate	++++	Phloroglucinol	+
Piperonal	++	Resorcinol	+
Quinone	+	Terpineol	+
Vanillin	+	Thymol	+

[a] Key: ++++, immediate persistent reaction with Dragendorff's reagent; +++, immediate, slight, persistent reaction; ++, immediate reaction, fading within 24 hr; and +, positive reaction only after 24 hr.

Table III—Reactions of Compounds with Alkaloidal Reagents

	Test Compounds[b]																					
	NACP		HACP		FACP		RuAc		QuAc		KmAc		ChAc		RhAc		Meth		Imp		Sant	
Reagent[a]	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Drag. sp.	+	+	+	−	+	−	+	−	+	−	+	−	+	−	−	−	+	−	+	−	+	−
Drag. pt.	+	+	+	−	+	−	−	−	−	−	+	−	−	−	−	−	+	−	+	−	−	−
Mayer's	+	+	+	−	+	−	+	−	+	−	+	−	−	−	−	−	+	−	+	−	−	−
Wagner's	+	+	+	−	+	−	+	−	+	−	+	−	−	−	−	−	+	−	+	−	+	−
STA	+	+	+	−	+	−	+	−	+	−	+	−	−	−	−	−	+	−	+	−	−	−
Kraut's	+	+	+	−	+	−	−	−	+	−	+	−	−	−	−	−	+	−	+	−	−	−
R. salt	+	+	+	−	+	−	−	−	±	−	+	−	+	−	+	−	+	−	+	−	−	−

[a] Key: Drag. sp., Dragendorff's spray reagent (Munier–Macheboeuf modification) (13); Drag. pt., Dragendorff's precipitation reagent (16); STA, silicotungstic acid; and R. salt, Reinecke salt. [b] Key: NACP, ammonia-catalyzed acetone condensation products; HACP, acid-catalyzed acetone condensation products; FACP, fixed alkali-catalyzed acetone condensation products; RuAc, acetylated rutin; QuAc, acetylated quercitin; KmAc, acetylated kaempferol; ChAc, acetylated chrysophanic acid; RhAc, acetylated rhein; Meth, methoxsalen; Imp, imperatorin; Sant, santonin; F1, fraction 1 (initial chloroform extract from acid medium); and F2, fraction 2 (purified chloroform extract from alkaline medium), as described under *Experimental*.

Acetone Condensation Products—The products (if any) from the interaction of acetone with concentrated hydrochloric acid, acetone with sodium hydroxide pellets, or acetone with concentrated ammonium hydroxide solution were prepared by keeping each pair in a closed flask for 3 days with frequent agitation. The dark-brown residue obtained with acetone and concentrated hydrochloric acid contained acid-catalyzed acetone condensation products, the viscid brown residue obtained with acetone and sodium hydroxide pellets contained fixed alkali-catalyzed acetone condensation products, and the dark, brittle, glassy solid residue obtained with acetone and concentrated ammonium hydroxide solution contained ammonia-catalyzed acetone condensation products.

Preparation of Nonalkaloidal Coupled Compounds with Dragendorff's Reagent—For IR analysis, complexes of khellin, imperatorin (ammidin), veratric acid, and cineole were prepared by dissolving the test compounds in aqueous acetic acid (20–50%, according to solubility) and adding dropwise Dragendorff's precipitation reagent. Khellin gave a heavy reddish-brown precipitate instantaneously, imperatorin gave a dark-brown crystalline compound after several hours, veratric acid gave an amorphous dark-brown deposit after sitting overnight, and cineole yielded a deep blood-red oil.

Reagents—Munier–Macheboeuf Dragendorff's spray reagent (13), Dragendorff's precipitation reagent (36), Mayer's reagent (36), Wagner's reagent (23), Kraut's reagent (37), 12% aqueous silicotungstic acid solution, and saturated Reinecke salt solution were used.

Test Procedure—The test compounds were dissolved in chloroform, alcohol, acetone, or chlorofrom–alcohol to make 0.2% solutions when solubility permitted or lower concentrations otherwise. Dissolution of very volatile liquids was not attempted to guard against loss of the test compound during solvent evaporation.

Aliquots, 10 μl, of individual test compounds were applied to microchromatoplates prepared by the slurry technique (32). Without attempting development, the spotted areas were sprayed with Dragendorff's reagent. The reaction results were recorded immediately (11). Spots of some very volatile liquids were examined by viewing the chromatoplates from the back (*e.g.*, isobutyraldehyde and isobutyl epoxide).

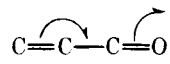
When certain constituents were extracted from preparations, two initial spots of the crude extract were cochromatographed using specified TLC systems (32–35). In one chromatogram, the constituents were located using specific chromogenic reagents (32–35) and then were used to monitor the second chromatogram that was sprayed with Dragendorff's reagent.

Partition of Test Compounds and Preparations—The purified total cardenolide fraction of *Acokanthera spectabilis* (29), the alcoholic extract of *L. dentata*, certain acetylated flavonoids [rutin, quercitin, kaempferol, hyperoside (3-D-galactoside hemipentahydrate), and quercitin glucuronide], the fixed alkali-catalyzed acetone condensation products, the acid-catalyzed acetone condensation products, the ammonia-catalyzed acetone condensation products, santonin, methoxsalen (xanthotoxin), and imperatorin were subjected to partition between acid–base and organic solvents. A concentrated alcoholic solution of the material was taken in dilute hydrochloric acid, the acidic solution was extracted with two portions of chloroform (fraction 1), and the aqueous phase was alkalinized with ammonia solution and reextracted with two portions of chloroform (fraction 2). Both fractions were tested with Dragendorff's spray and precipitation reagents, Mayer's reagent, Wagner's reagent, silicotungstic acid solution, Reinecke salt solution, and Kraut's reagent.

Recording Results—If a very slight or no color was observed with the spray reagent, larger quantities of the test solution (20–50 μl) were retested. Test compounds and results are given in Tables I and II; Table III gives the results of the partition experiments.

RESULTS AND DISCUSSION

Of the many organic compounds tested, only positively reacting compounds are given in Table I. Compounds reported by Farnsworth *et*



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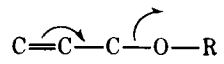
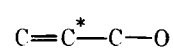


II

al. (3) to give a positive reaction are given in Table II after being categorized according to whether they conform to the structural requirements determined by these investigators.

The structures of 86 positively reacting compounds (Table I) and some of the 55 compounds reported by Farnsworth *et al.* (3) (Table II) were



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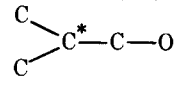
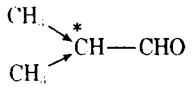


IV

studied. Many of the compounds were conjugated aldehydes, ketones, and lactones, thus fulfilling the minimal structural requirement indicated by Farnsworth *et al.* (3). These compounds are listed in column A of Tables I and II. Many other compounds lack these structural features (column B of Tables I and II).

Compounds of column B (Table I) have no common structural feature that correlate them to compounds of column A. While some have carbonyl or lactone functions (the first eight compounds), they lack conjugation to these functions. All other compounds of column B lack carbonyl or lactone functions. Thus, neither these features nor the conjugation is necessary for the reaction.

All of the positively reacting compounds are oxygenated, and the oxygen represents a site of high electron density—*viz.*, carbonyl, lactone,



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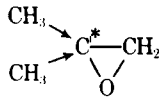

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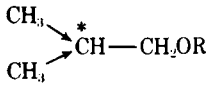

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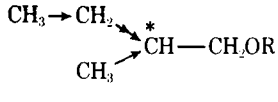

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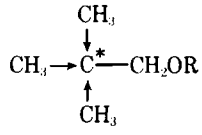

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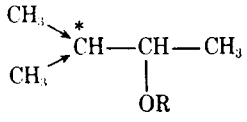

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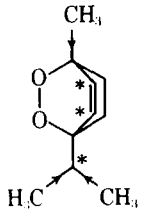

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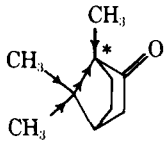

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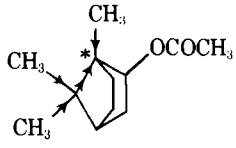

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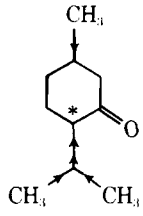

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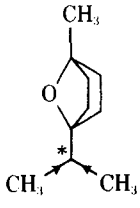

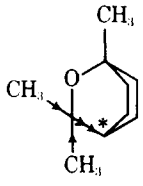

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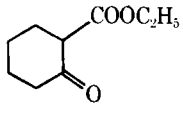
XVII



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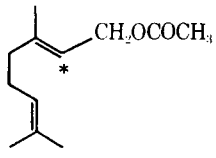

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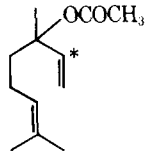

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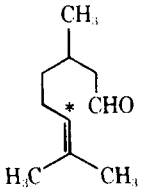

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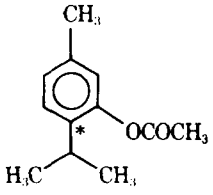

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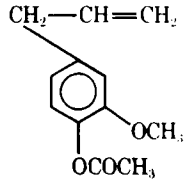

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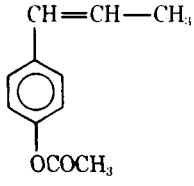

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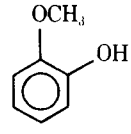

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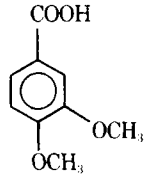

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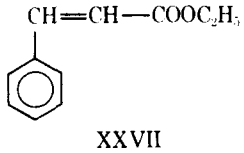

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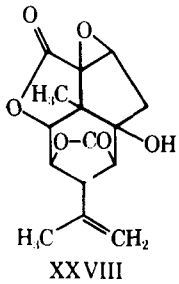

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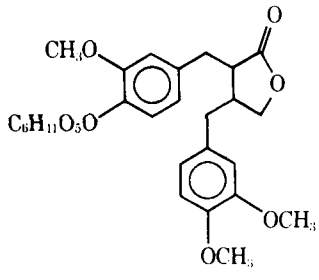

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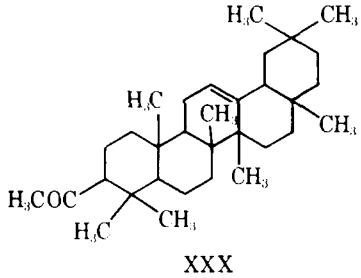

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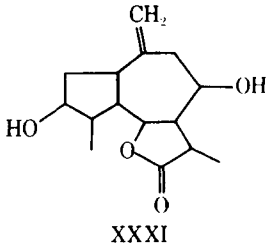

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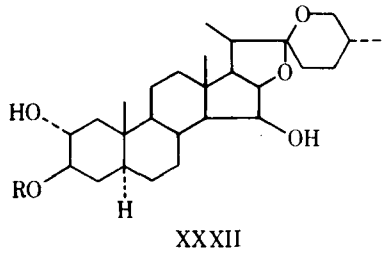

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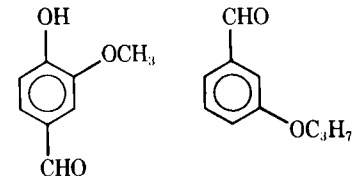

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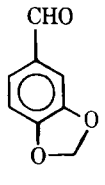

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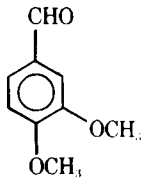

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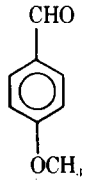

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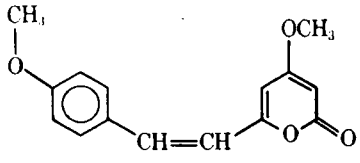

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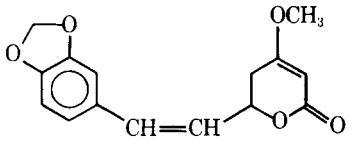

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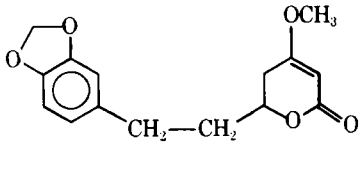

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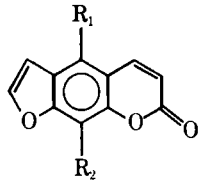

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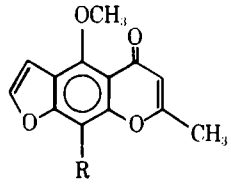

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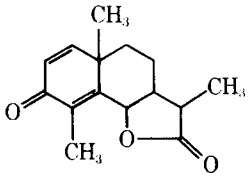

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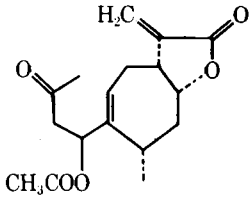

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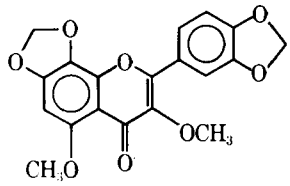

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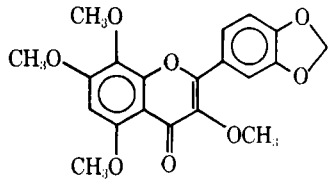

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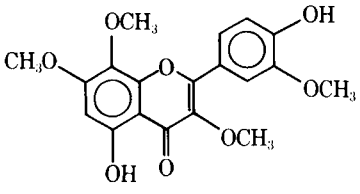

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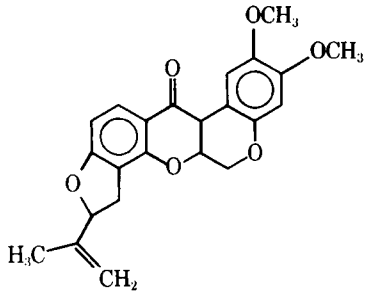

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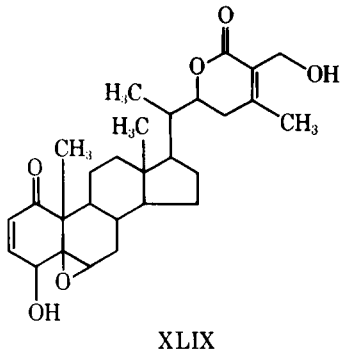

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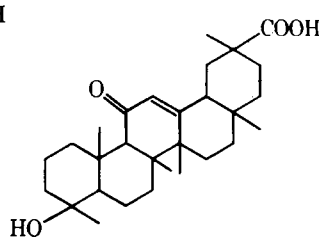


L

ether, ester, epoxide, and peroxide. This observation suggested that: (*a*) it may not be the carbonyl function *per se* but instead the high availability of its electrons that accounts for the reaction, and (*b*) other oxygen functions of comparably high electron density may react similarly. This suggestion has been realized by the strong positive reactions of some conjugated ethers and esters (Table I) and the positive reaction of some α,β-unsaturated alcohols and acids. The existence of the α,β-unsaturation in these compounds would increase substantially the electron density at the oxygen function (I and II), rendering it sufficiently basic to form coordinate complexes with Lewis acids (alkaloidal reagents).

The compounds studied lacking any conjugated carbonyl or lactone function were V, isobutyraldehyde; VI, isobutyl epoxide; VII, isobutyl ether (R = alkyl) or ester (R = acyl); VIII–X esters (R = acyl) or ethers (R = alkyl): VIII, active isopentyl ethers or esters; IX, neopentyl ethers or esters; and X, isopropylmethyl ethers or esters; XI, ascaridole; XII, camphor; XIII, bornyl acetate; XIV, menthone; XV, 1,4- and 1,8-cineoles; XVI, 2,3-dihydro-4-pyran; XVII, 5,6-dihydro-2-pyran; XVIII, ethyl-2-cyclohexanone carboxylate; XIX, geranyl acetate; XX, linalyl acetate; XXI, citronellal; XXII, thymol acetate; XXIII, acetyleugenol; XXIV, acetyl anethole; XXV, guaiacol; XXVI, veratric acid; XXVII, ethyl cinnamate; XXVIII, picrotoxinin; XXIX, arctiin; XXX, acetyl-β-amyrin; XXXI, lipidiol; and XXXII, digitonin (R = sugar moiety).

Many compounds that lack α,β-unsaturation (to the oxygen) react positively (Tables I and II). Nonetheless, in almost all of these compounds, the β-carbon (denoted by an asterisk) bears one or more free alkyl groups. The electron-repelling methyl groups increase the electron density at the β-carbon, resulting in the same effect as does α,β-unsaturation. In V–XV, the effect of the free alkyl groups is demonstrated; the arrowheads in the structural formulas of these compounds qualitatively represent the electron-repelling effect of these groups.

These observations were significant in establishing III or IV as the structural feature necessary for the false alkaloid reaction, provided that the high availability of electrons at the oxygen was not altered by other functions.

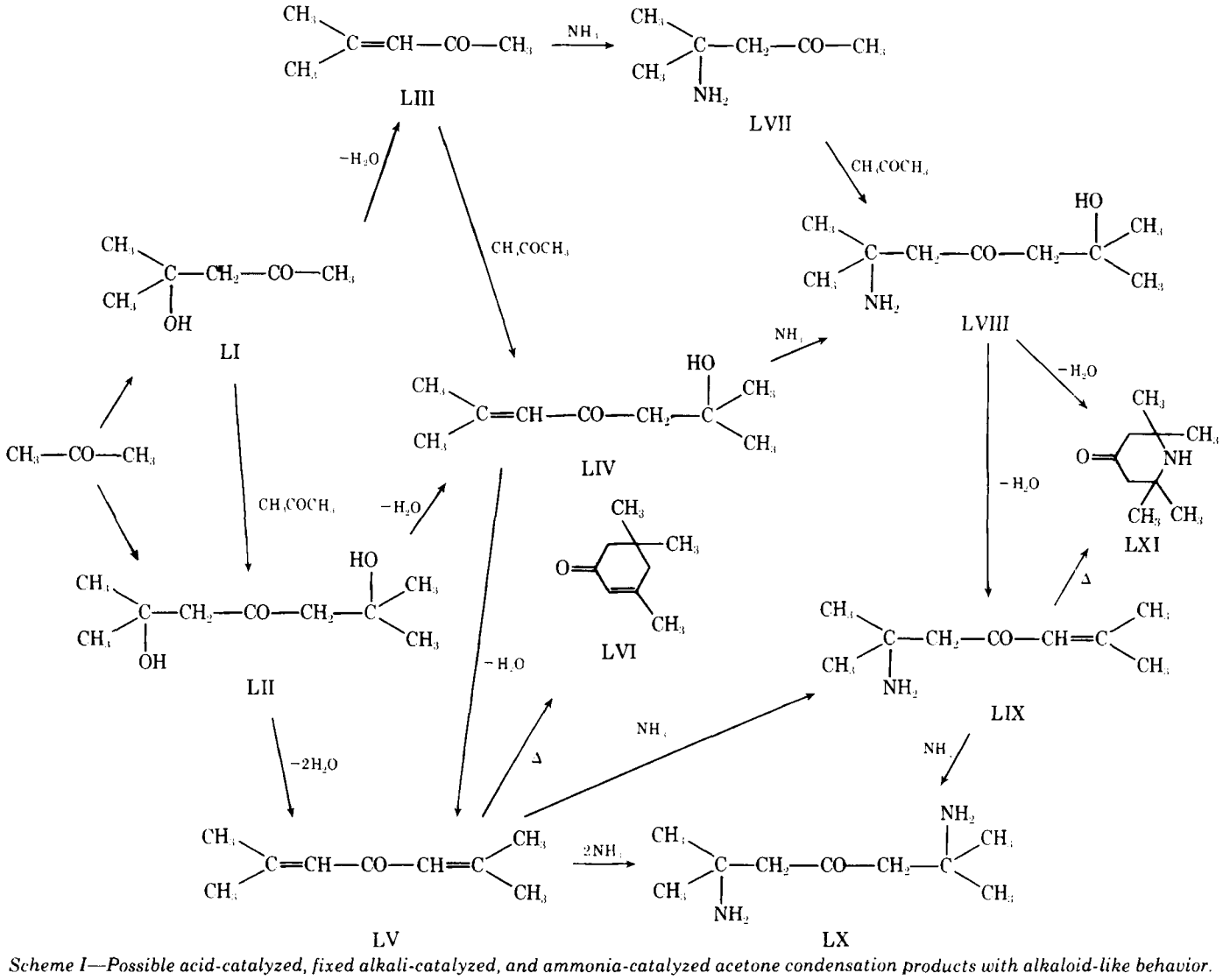
The β-carbon (denoted by an asterisk) of III may constitute a part of an aromatic structure. The oxygen function in III and IV may be an aldehyde, a ketone, a lactone, an alcoholic or phenolic ether, an ester, an oxide, or a peroxide.

Structures III and IV were demonstrated to fulfill the requirements of the test by the typical alkaloid-like reactions given by acrolein, crotonaldehyde, citral, carvone, XI, XIX, piperonal (XXXV), and veratraldehyde (XXXVI) (General Structure III) and by V–XV (General Structure IV).

If the oxygen of the essential structure is an alcohol, a phenol, or a hemiacetal, a negative or slight reaction is given, *e.g.*, geraniol, terpineol, linalool, borneol, isoborneol, primary isobutanol, pyrocatechol, resorcinol, pyrogallol, orcinol, hydroquinone, guaiacol, benzhydrol, thymol, α-amyrin, lanosterol, salicyl alcohol, isopentanol, and cinnamyl alcohol. Esterification of these alcohols and phenols in many instances gave positively reacting esters (Table I).

Carboxylic acids also were expected to react negatively due to their low availability of electrons at the carboxylic group or because acids tend to associate as cyclic dimers (39). Thus, crotonic, anisic, maleic, acrylic, benzoic, cinnamic, *p*-hydroxybenzoic, glycyrrhizic, glycyrrhetinic, and quillaic acids gave negative tests, despite the fulfillment of the structural requriement. Maleic anhydride, phthalic anhydride, and cantharidin also gave a negative reaction.

The reaction of compounds that fulfilled the essential requirement was affected by other functions that altered the electron density at the oxygen or the β-carbon. Thus, when the β-carbon was involved in an aromatic structure or a conjugated double bond system, electron-repelling substituents enhanced the reaction, *e.g.*, pyrocatechol (−) [*cf.*, guaiacol (±)



Scheme I—Possible acid-catalyzed, fixed alkali-catalyzed, and ammonia-catalyzed acetone condensation products with alkaloid-like behavior.

and eugenol (+++)], *m*-cresol (−) [*cf.*, thymol (+)], anisole (−) [*cf.*, anethole (+)], and benzaldehyde (−) and 3-hydroxybenzaldehyde (−) [*cf.*, anisaldehyde (++), veratraldehyde (+++), piperonal (+++), and 3-butoxybenzaldehyde (++)]. On the other hand, an electron-attracting substituent decreased or even eliminated the reaction, *e.g.*, acetophenone (+++) and *p*-aminoacetophenone (+++) [*cf.*, *p*-nitroacetophenone (−) and *p*-hydroxyacetophenone (−)].

The strong reactions given by 3-butoxybenzaldehyde, piperonal, veratraldehyde, eugenol, and acetyleugenol in contrast to the negative reactions given by benzaldehyde, 3-hydroxybenzaldehyde, and vanillin mean that the reaction of the former five compounds should not be attributed wholly to the conjugated carbonyl structure but should partially be attributed to their conjugated ether and/or ester functions. The same also applies to conjugated carbonyl compounds and lactones with additional reactive ether, ester, or carbonyl functions: XXXIII, vanillin; XXXIV, 3-butoxybenzaldehyde; XXXV, piperonal; XXXVI, veratraldehyde; XXXVII, anisaldehyde; XXXVIII, yangonin; XXXIX, methysticin; XL, dihydromethysticin; XLI, psoralen (furocoumarin) ($R_1 = R_2 = H$), bergapten ($R_1 = OCH_3$), methoxsalen ($R_2 = OCH_3$), imperatorin (R_2 = isoamyleneoxy), and isopimpinellin ($R_1 = R_2 = OCH_3$); XLII, furochromones khellin (R = OCH_3) and visnagin (R = H); XLIII, santonin; XLIV, xanthumin; XLV, meliternatin; XLVI, meliternin; XLVII, ternatin; XLVIII, rotenone; XLIX, withaferin A; and L, glycyrrhetinic acid.

The IR spectra of khellin, imperatorin, and veratric acid provided evidence that both the carbonyl and ether functions in these compounds are involved in the reaction. The strong band assigned to the carbonyl function (1655–1620 cm^{-1} in khellin, 1710–1650 cm^{-1} in imperatorin, and 1685–1655 cm^{-1} in veratric acid) were distorted and split in the spectra of the corresponding coupled compounds. More pronounced changes were observed in the region of the ether and aralkyl ether stretching vibration; thus, the strong bands at 1085 cm^{-1} in khellin and 1090 cm^{-1} in imperatorin were split into doublets of medium intensity in the spectra of the coupled compounds. The band at 1095 cm^{-1} with veratric acid appeared only as a shoulder in the spectrum of the coupled compound. The bands between 1000 and 979 cm^{-1} (–C=CH deformation) also were affected, *i.e.*, weakened and shifted toward a higher frequency in the spectra of the coupled compounds.

The reactivity of compounds with the essential structure was eliminated almost completely by hydroxyl groups in their structures. The hydroxyl groups competed for the oxygen function of the essential structure, through inter- and/or intramolecular hydrogen bonding. This competition may explain the contrast in the reaction of acetophenone (+++) *versus* *p*-hydroxyacetophenone (−), coumarin (+++) *versus* umbelliferone (±), anisaldehyde (+++) *versus* vanillin (±), digitoxin (+++) and strophanthin (++) *versus* ouabain (G-strophanthin) (+), menadione (++++) *versus* 3-hydroxymenadione (−), and santonin XLIII (+++) and xanthumin XLIV (+++) *versus* lipidiol XXXI (−) and isolipidiol (±).

Therefore, naturally occurring hydroxylated flavonoids and hydroxyanthracene derivatives were expected to react negatively. Acetylated derivatives of these compounds gave positive tests with Dragendorff's spray reagent (Table I) as well as with some other alkaloidal precipitants (Table III). Acetylation and alkylation not only eliminated the interfering action of the hydroxyls but also introduced additional conjugated ester or ether functions; thus, the alkaloid-like reaction was intensified.

Briggs and Locker (8) reported that XLV–XLVII, three alkylated hydroxyflavones, gave precipitates with the usual alkaloid reagents and gave crystalline salts with acids. This alkaloid-like behavior was attributed to the fact that each molecule was completely alkylated. However,

Farnsworth (4) stated that the reaction was due to the conjugated carbonyl function and that other flavonoids would react similarly. The present work shows both of these reports to be partially correct; despite the conjugated carbonyl nature, flavonoids do not show alkaloid-like behavior unless they are almost completely alkylated.

Being alkylated isoflavonoids, all rotenoids presumably give positive alkaloid reactions; *e.g.*, rotenone (+++).

Housholder and Camp (40) stated that treatment of plant material with acetone and ammonia solution can give rise to alkaloid-like artifacts. These investigators were unable to identify the condensation products of the reaction, but they reported that the reaction rate was enhanced by light and the atmosphere.

Upon wet packing a column of silica gel with benzene–acetone–ammonia and keeping it overnight prior to the application of any material, discoloration of the column was observed[2]. Draining and evaporating the brown solvent mixture yielded a brown residue, which gave typical alkaloid reactions with Dragendorff's spray reagent as well as with other alkaloid precipitants. Neither benzene nor the plant material but instead acetone and/or ammonia apparently were responsible for the production of the alkaloid-like artifacts because both were common factors.

These artifacts may be either nitrogenous condensation products of acetone and ammonia or nonnitrogenous, alkali-catalyzed aldol condensation products of acetone that fulfill the requirements of the false reaction.

Acid-catalyzed, fixed alkali-catalyzed, and ammonia-catalyzed acetone condensation products gave a strong positive reaction with Dragendorff's spray reagent. TLC revealed three main, qualitatively identical Dragendorff-positive spots in acid-catalyzed and fixed-alkali-catalyzed acetone condensation products at R_f 0.78, 0.67, and 0.55 on silica gel in chloroform–methanol (93:7) and at R_f 0.65, 0.53, and 0.42 on silica gel in chloroform–methanol–acetic acid (89:10:1), respectively. The ammonia-catalyzed acetone condensation products revealed at least seven spots, including three identical to those of the acid-catalyzed and fixed alkali-catalyzed condensation products. The additional four spots were at low R_f values: 0.32, 0.21, 0.11, and 0.00 in chloroform–methanol and 0.26, 0.45, 0.05, and 0.00 in chloroform–methanol–acetic acid. An eighth spot, R_f 0.50 in chloroform–methanol and 0.46 in chloroform–methanol–acetic acid, gave a typical pink color.

Obviously, the spots common in the acid-catalyzed, fixed alkali-catalyzed, and ammonia-catalyzed acetone condensation products were of nonnitrogenous compounds. This conclusion indicates the compounds to be aldol condensation products of acetone. Scheme I shows the possible reaction products of acetone with acids, fixed alkalis, and ammonia. Compounds LIII–LVI are α,β-unsaturated ketones that fulfill the structural requirement of the false reaction. The additional low R_f spots in the ammonia-catalyzed acetone condensation products were assumed to be amino compounds (LVII–LXI) that arise from the condensation of ammonia with the reactive α,β-unsaturated ketones LIII–LV. This assumption was favored by the positive nitrogen test given by the ammonia-catalyzed acetone condensation products. Further evidence for the postulated reaction sequence was the development of the additional low R_f spots, absent from the acid-catalyzed and fixed alkali-catalyzed condensation products, when the latter two were kept with concentrated ammonia solution overnight.

From the results of the partition experiments (Table III), nonnitrogenous Dragendorff-positive plant constituents apparently do not interfere with alkaloid detection and isolation if necessary partition purification steps are performed. The nitrogenous acetone–ammonia reaction products react typically as alkaloids and interfere seriously with alkaloid detection and/or isolation.

REFERENCES

(1) C. C. Fulton, *Am. J. Pharm.*, **104**, 244 (1932).

(2) R. Martello and N. R. Farnsworth, *Lloydia*, **25**, 176 (1962).

(3) N. R. Farnsworth, N. A. Pilwski, and F. J. Draus, *ibid.*, **25**, 312 (1962).

(4) N. R. Farnsworth, *J. Pharm. Sci.*, **55**, 225 (1966).

(5) L. Rosenthaler, "The Chemical Investigation of Plants," G. Bell and Sons Ltd., London, England, 1930, pp. 25, 26.

(6) C. O. Wilson and O. Gisvold, "Text Book of Organic Medicinal and Pharmaceutical Chemistry," 3rd ed., Lippincott, Philadelphia, Pa., 1956, p. 242.

(7) A. R. Furgiuele, Ph.D. dissertation, University of Pittsburgh, Pittsburgh, Pa., 1962, p. 100; through Ref. 3.

(8) L. H. Briggs and R. H. Locker, *J. Chem. Soc.*, **1949**, 2157.

(9) R. F. Raffauf, *Econ. Bot.*, **16**, 171 (1962).

(10) D. Kraft, *Pharmazie*, **8**, 170 (1956).

(11) G. K. Nikonov and A. I. Ban'Kovskii, *Tr. Vses. Nauchn-Issled. Inst. Lekarstv. Aromat. Rast.*, No. 11, 296 (1959); through *Chem. Abstr.*, **56**, 10490 (1962).

(12) N. R. Farnsworth and K. L. Euler, *Lloydia*, **25**, 186 (1962).

(13) R. Munier and M. Macheboeuf, *Bull. Soc. Chem. Biol.*, **33**, 846 (1951).

(14) E. Tyihak, *J. Chromatogr.*, **14**, 125 (1964).

(15) T. Thies and F. W. Reuther, *Naturwissenschaften*, **41**, 230 (1954).

(16) K. Thoma, R. Rombach, and E. Ulimann, *Sci. Pharm.*, **32**, 216 (1964).

(17) H. M. Bregoff, E. Roberts, and C. C. Delwiche, *J. Biol. Chem.*, **205**, 565 (1953).

(18) R. Munier, *Bull. Soc. Chem. Biol.*, **35**, 1225 (1953).

(19) H. Jatzkewitz and Z. Hoppe-Seylera, *Physiol. Chem.*, **292**, 99 (1953); through Ref 32.

(20) D. Vagujfalvi, *Planta Med.*, **8**, 34 (1960).

(21) V. H. C. Menben and G. Stopel, *ibid.*, **24**, 8 (1973).

(22) M. E. Wall, C. S. Fenske, J. W. Garvin, J. J. Willaman, Q. Jones, B. G. Schubert, and H. S. Gentry, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 695 (1959).

(23) E. Abisch and T. Reichstein, *Helv. Chim. Acta*, **43**, 1844 (1960).

(24) X. A. Domingues, *Econ. Bot.*, **14**, 157 (1960).

(25) W. H. Tallent, V. L. Stromberg, and E. C. Horning, *J. Am. Chem. Soc.*, **77**, 6361 (1955).

(26) K. L. Euler and N. R. Farnsworth, *Lloydia*, **25**, 296 (1962).

(27) L. A. Anderson, N. S. Dogget, and M. S. F. Ross, *Planta Med.*, **32**, 2, 125 (1977).

(28) Y. A. Mohamed, "Proceedings of the 10th Science Week," Damascus, Syrian Arab Republic, part 1, 1969, p. 1.

(29) M. S. Karawya, S. M. Abdel-Wahab, and H. M. Niazi, *Planta Med.*, **24**, 234 (1973); M. S. Karawya, S. M. Abdel-Wahab, and H. M. Niazi, *Egypt. J. Pharm. Sci.*, **15**, 33 (1974).

(30) A. A. M. Habib and T. S. Sarg, *Bull. Fac. Sci., Riyadh Univ.*, **8**, 341 (1977).

(31) M. M. A. Hassan, A. A. M. Habib, and F. J. Muhtadi, *Pharmazie*, **31**, H9 (1976).

(32) E. Stahl, "Thin-Layer Chromatography," 2nd ed., Springer-Verlag, Berlin, Germany, 1969.

(33) K. Macek, "Pharmaceutical Application of Thin-Layer and Paper Chromatography," Elsevier, Amsterdam, The Netherlands, 1972.

(34) E. G. C. Clark, "The Isolation and Identification of Drugs," vols. I and II, Pharmaceutical Press, London, England, 1969 and 1975.

(35) "Official Methods of Analysis," 12th ed., Association of Official Analytical Chemists, Washington, D.C., 1975.

(36) A. K. Kiang and B. Douglas, Proc. Third Congress Pan Indian Ocean Scientific Association, Tananarive, Section G, 1957, p. 19; through Refs. 4 and 12.

(37) C. P. Stewart and A. Stolman, "Toxicology," vol. 2, Academic, New York, N.Y., 1961.

(38) A. A. M. Habib and A. M. Metwally, *Planta Med.*, **23**, 88 (1973).

(39) I. L. Finar, "Organic Chemistry," vol. I, 6th ed., Longman, London, England, 1973.

(40) D. E. Housholder and B. J. Camp, *J. Pharm. Sci.*, **54**, 1676 (1965).

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