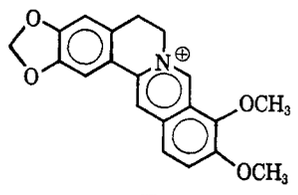
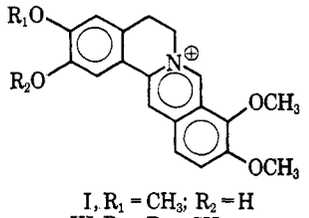
Recently, we described the first efficient separation of columbamine and the related alkaloids berberine (II), palmatine (III), and jatrorrhizine (IV), as well as the characterization for the first time of pure columbamine (as the crystalline chloride and iodide) from natural sources.[2] Previous to this report, columbamine had been isolated in pure form only as its tetrahydro derivative.[3] We wish now to report a procedure for the conversion of berberine (II) into columbamine (I). Since the total synthesis of berberine has already been carried out,[3] this conversion constitutes a formal total synthesis of columbamine. Of greater importance, this synthesis should prove to be of considerable practical value to phytochemists who require authentic comparison samples of columbamine salts, particularly in view of the fact that berberine salts are articles of commerce.

The conversion of berberine into columbamine was carried out using a simple two-step process. In the first step, commercial berberine (II) was demethylenated by sulfuric acid in the presence of phloroglucinol.[4] In the second step, the resulting demethyleneberberine (V) was subjected to partial methylation by dimethyl sulfate in the presence of sodium bicarbonate, and the methylation products were separated as the chlorides by chromatography on acid alumina as described previously for the natural Columbo root alkaloids.[2]

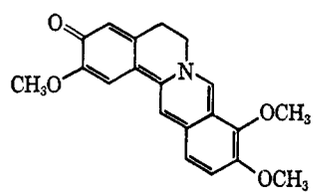
The critical factor in the successful partial methylation of V to columbamine is control of the basicity of the reaction medium. Both jatrorrhizine (IV) and demethyleneberberine (V) give a red coloration in sodium bicarbonate solution, a reaction which is not shown by columbamine (I); in the case of jatrorrhizine, the red color has been attributed to the anion, one canonical form of which is a quinonoid structure (VI).[2] It is apparent that columbamine (I), which cannot form an anion stabilized by a quinonoid contributor, should be a weaker acid than either IV or V. This prediction was confirmed experimentally by pK_a determinations: the pK_a of columbamine (8.54) is appreciably higher than that of either jatrorrhizine (7.09) or demethyleneberberine (7.29); as expected, the latter compound is methylated, albeit very slowly, in an aqueous sodium bicarbonate solution at 40°. After 8 hr, methylation takes place to the extent of about 30%, the methylation products consisting of columbamine, jatrorrhizine, and palmatine in a ratio of about 8:3:1; the yield of columbamine from V in this reaction, which probably could be run to advantage for a longer reaction period, is 19%.



II



I, $R_1 = CH_3$; $R_2 = H$
III, $R_1 = R_2 = CH_3$
IV, $R_1 = H$; $R_2 = CH_3$
V, $R_1 = R_2 = H$



VI

Experimental Section

pK_a Measurements.—The chlorides of I, IV, and V were dissolved in distilled water, acidified with standard hydrochloric acid, and back titrated with standard aqueous hydroxide. Changes in pH were followed using a Model G Beckman pH meter. End points were determined by plotting dpH/dV against volume of base added.

Controlled Methylation of Demethyleneberberine.—Demethyleneberberine iodide (0.2 g)[4] was converted into the corresponding chloride by passing its solution in 1:1 acetone–water through a column (1 × 8 cm) of Amberlite IR-A 410 resin (chloride form), which was washed in 1:1 acetone–water prior to use.

A mixture of demethyleneberberine chloride (0.1597 g), water (50 ml), dimethyl sulfate (1 ml), and saturated aqueous sodium bicarbonate (5 ml) was stirred at 40° for 8 hr; additional dimethyl sulfate and sodium bicarbonate were added as required during this time in order to assure an excess of these reagents during the reaction. The reaction solution was concentrated, the alkaloids were precipitated as the iodides, and the iodides were converted to the chlorides using Amberlite IR-A 410 resin. The mixed chlorides were chromatographically resolved using Woelm acid alumina as described previously for the naturally occurring compounds.[2] Unreacted demethyleneberberine was not recovered, owing to the tenacity with which it is adsorbed on alumina.

The following yields of alkaloids were obtained, the total representing about a 30% conversion of demethyleneberberine methylated products. All products were identified by melting point and by infrared spectra comparison with pure alkaloids of natural origin. (See Table I.)

TABLE I

Column eluent	Product (as chloride)	Yield, mg
4% methanol in chloroform	Palmatine	4.0
5.5–8% methanol in chloroform	Columbamine	31.3
12–15% methanol in chloroform	Jatrorrhizine	11.8

Registry No.—I, 1355-76-6; II, 2086-83-1.

Acknowledgment.—This investigation was supported by Public Service Research Grant RG-5640, National Institutes of Health.

(2) M. P. Cava, T. A. Reed, and J. L. Beal, *Lloydia*, **28**, 73 (1965).
(3) R. H. F. Manske and W. R. Ashford, "The Alkaloids," Vol. IV, R. H. F. Manske and H. L. Holmes, Ed., Academic Press Inc., New York, N. Y., 1954, Chapter 29.
(4) E. Späth and H. Quietensky, *Ber.*, **58**, 2267 (1925).

Reaction of Diimide N-Oxide Derivatives and Grignard Reagents. Evidence for Radical Intermediates[1]

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During the studies on the synthesis of azoxy compounds from the reaction of Grignard reagents and

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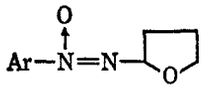
TABLE I

N-ARYL-N′-(2-TETRAHYDROFURYL)DIIMIDE N-OXIDES



Ar	Registry no.	Empirical structure	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found
Phenyl	7781-67-1	$C_{10}H_{12}N_2O_2$	62.48	62.38	6.29	6.29	14.58	14.46
p-Tolyl	7781-68-2	$C_{11}H_{14}N_2O_2$	64.06	64.36	6.84	7.11	13.58	13.46
p-Chlorophenyl	7781-69-3	$C_{10}H_{11}N_2O_2Cl$	52.99	53.02	4.89	5.40	12.36	11.87

TABLE II

REACTION OF AZOXY COMPOUNDS AND GRIGNARD REAGENTS

Azoxy compound, Ar—N(O)=N—X: Ar	X	mmoles	Grignard, "RMgX": R	mmoles	Yield, %: Arylazoxy alkane	Yield, %: Arylazoxy ether
Phenyl	OTs	20	*n*-Butyl	24	25	6
Phenyl	OTs	20	Ethyl	25	21	9
p-Tolyl	OTs	10	Ethyl	12	29	14
p-Chlorophenyl	OTs	11	*n*-Butyl	12	29	9
Phenyl	F	3.6	Methyl	5	15	27
p-Chlorophenyl	F	2.9	Methyl	4.2	46	14

arylnitrosohydroxylamine tosylates[2] or aryl-N′-fluorodiimide N-oxides,[3] it was observed that reactions conducted in tetrahydrofuran (THF) solutions often led to undesirable, unidentified by-products. At that time this difficulty was overcome by using methylene chloride instead of THF as the reaction solvent for the aliphatic Grignard reagents.[2] Now the structure of the major by-product of the reaction in THF has been found to be the N-aryl-N′-(2-tetrahydrofuryl)diimide N-oxides (**2**).

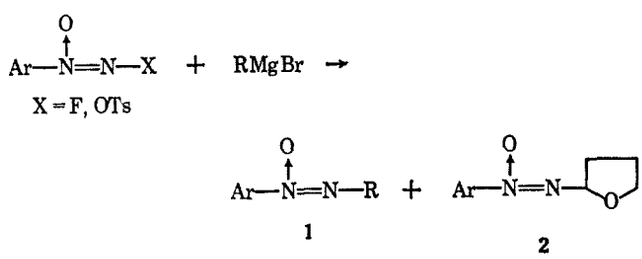
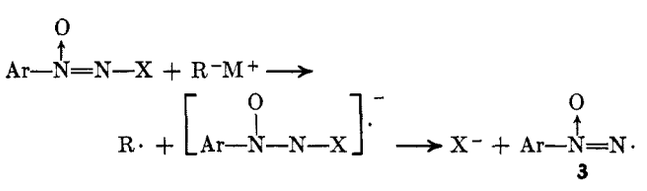


Table I summarizes the analytical data for three examples of azoxy compounds **2**; Table II indicates the yields of azoxyalkane and azoxy THF materials obtained in representative experiments.

Structures **2** are based on the empirical formulas of Table I and the following spectra data. Infrared spectra of the N-aryl-N′-(2-tetrahydrofuryl)diimide N-oxides (**2**) were similar to those of N-aryl-N′-alkydiimide N-oxides (**1**); both had strong 6.73–6.76 μ absorption characteristics of the diimide N-oxide (azoxy) group.[4] The ultraviolet spectrum of **2**, Ar = phenyl, had λ_{max} (cyclohexane) 247 mμ (ϵ_{max} 7300).[5] Also, the proton nmr spectra of the samples of **2** were consistent with the assigned structure. The single proton at C-2 in the THF ring was observed as a multiplet at 335–350 cps,[6] the four C-3 and C-4 protons were an envelope at 100–150 cps, and the two C-5 protons were a complex triplet centered at δ 3.98.

The formation of significant quantities of products such as **2** in the reactions indicates that a radical process may be occurring. In view of the observations that, of the several nucleophiles examined, only the Grignard reagent effected carbon–nitrogen-bond formation with the arylnitrosohydroxylamine tosylates[2] (other nucleophiles displaced the nitrosohydroxylamine anion by attack on sulfur) and that the N-fluorazoxy compounds were inert to nucleophiles other than organometallics, it is tempting to rationalize the results in the terms sketched below. Thus, electron transfer from the organometallic to the azoxy compound could, by way of the radical-anion intermediate,[7] lead to an aliphatic radical and a radical species such as **3**.[8,9] Radical abstraction of hydrogen from THF would then lead to the 2-tetrahydrofuryl radical. It is possible that both **1**



(2) T. E. Stevens, *J. Org. Chem.*, **29**, 311 (1964). Recently, attempts to repeat preparations of aromatic azoxy compounds by this method in THF solution often failed; however, preparations with methylene chloride–ether as solvent worked satisfactorily. It is recommended that the methylene chloride solvent system be used for all preparative experiments.

(3) T. E. Stevens and J. P. Freeman, *ibid.*, **29**, 2279 (1964).

(4) J. P. Freeman, *ibid.*, **27**, 1309 (1962); **28**, 2508 (1963).

(5) Ultraviolet absorption data on related structures includes λ_{max} 243 mμ (ϵ_{max} 8400) for N-phenyl-N′-fluorodiimide N-oxide[3] and λ_{max} 246 mμ (ϵ_{max} 10,300) for N-phenyl-N′-butyldiimide N-oxide.[4]

(6) Spectra were measured in carbon tetrachloride on a Varian Associate A-60 spectrometer. Values in cycles per second are downfield from internal tetramethylsilane.

(7) The occurrence of electron transfer from *n*-butylmagnesium bromide to related electron acceptors has been demonstrated: G. A. Russell, E. G. Janzen and E. T. Strom, *J. Am. Chem. Soc.*, **86**, 1807 (1964). Also see D. Seyferth and B. Prokai, *J. Org. Chem.*, **31**, 1702 (1966).

(8) However, efforts to find evidence for **3** by means of electron spin resonance methods have not been successful: Dr. K. J. Martin, personal communication.

(9) The arylnitrosohydroxylamine tosylates[2] are unstable thermally and appear to decompose to give a species such as **3**, but in the thermal experiments **3** decomposes to give products (phenyl radicals and nitrogen) that apparently arise from the diazotate radical C_6H_5—N=N—O·: E. A. Dorko and T. E. Stevens, *Chem. Commun.*, 871 (1966).

and **2** arise from radical coupling[10] or radical displacement reactions, but evidence other than the isolation of **2** is not at hand at this time.

Experimental Section

The usual procedures[2,3] were used to obtain the results shown in Table II. An example follows.

Reaction of Ethyl Grignard Reagent and N-phenyl-N'-(*p*-toluenesulfonoxy)diimide N-Oxide.—To a solution of 5.84 g (20 mmoles) of the above tosylate in 100 ml of THF in a nitrogen atmosphere was added 8 ml of 3 *M* ethyl magnesium bromide in ether (Arapahoe Chemicals). The ether was swept out in the nitrogen stream and the solution was heated at 55° for 5 hr. After the usual hydrolysis procedure,[2] the organic residue was chromatographed on silica gel. Pentane–methylene chloride (2:1) eluted 0.57 g of N-phenyl-N'-ethyldiimide N-oxide; methylene chloride eluted 0.30 g of starting tosylate; and methylene chloride–ethyl acetate eluted 0.30 g of N-phenyl-N'-(2-tetrahydrofuryl)diimide N-oxide, a yellow oil.

(10) Such a mechanism would be similar to that reported for some carbon alkylations of nitro benzyl halides by 2-nitropropane salts: R. C. Kerber, G. W. Urry, and N. Kornblum, *J. Am. Chem. Soc.*, **87**, 4520 (1966). We have not observed any significant amounts of radical coupling products in reactions of arylnitrosohydroxylamine tosylates or N'-fluorodiimide N-oxides and the lithium salt of 2-nitropropane. The N-fluoroazoxy materials did not react in these experiments, and, with the tosylate, 2,3-dimethyl-2,3-dinitrobutane (traces) appeared only at temperatures where thermal decomposition[9] may have given radical products.

Syntheses of 6-Deoxy-2,4-di-*O*-methyl-D-galactose (Labilose) and of 2,4-Di-*O*-methyl-D-galactose

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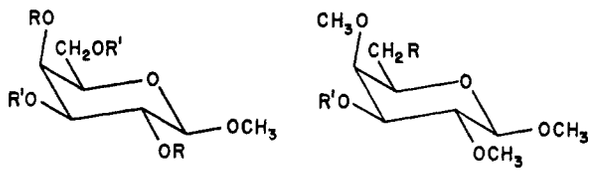
Labilomycin, an antibiotic which inhibits the growth of Gram-positive bacteria[2] and which is effective against certain tumor cells,[3] is produced by the microorganism *Streptomyces albosporeus*.[4] The antibiotic contains, as part of its structure,[5] a methylated sugar termed labilose, which has been shown[6] to be 6-deoxy-2,4-di-*O*-methyl-D-galactose. The preparation, in these laboratories, of methyl 3,6-di-*O*-mesyl-β-D-galactopyranoside (**1**)[7] (Chart I) in one stage from methyl β-D-galactopyranoside provided a convenient starting material for the synthesis of labilose.

Methylation of **1** gave methyl 3,6-di-*O*-mesyl-2,4-di-*O*-methyl-β-D-galactopyranoside (**2**)[7] which was reduced with lithium aluminum hydride in anhydrous tetrahydrofuran. Instead of the expected methyl β-D-labiloside, a mixture of methyl 2,4-di-O-methyl-β-D-galactopyranoside (**3**) and methyl 3,6-anhydro-2,4-di-*O*-methyl-β-D-galactopyranoside (**4**) was produced. The physical constants of both of these compounds agreed with those in the literature. The identity of **3** was confirmed by remesylation to the starting material (**2**). The synthesis of **3** as above, followed by hydrolysis to the free sugar, gave an alternative synthesis to that of Jeanloz[8] for 2,4-di-*O*-methyl-D-galactose which has been isolated several times[9-11] from the hydrolysis of methylated polysaccharides.

An attempt to prepare methyl 2,4,6-tri-*O*-methyl-β-D-galactopyranoside by treatment of **2** with sodium methoxide in methanol, as previously described,[12,13] gave a mixture of compounds indicating that nucleophilic attack at the C-6 atom is inhibited.[14] A similar inhibition would account for the results obtained in the above reduction.

The synthesis of methyl β-D-labiloside was accomplished by reduction of the intermediate 6-iodo compound. Treatment of **2** with sodium iodide in boiling methyl ethyl ketone effected displacement of the 6-mesyloxy group. Methyl 6-deoxy-6-iodo-3-*O*-mesyl-2,4-di-*O*-methyl-β-D-galactopyranoside (**5**) was isolated from the reaction mixture in 59% yield by silica gel column chromatography. Treatment of the 6-iodo derivative (**5**) with lithium aluminum hydride in anhydrous tetrahydrofuran gave crystalline methyl 6-deoxy-2,4-di-*O*-methyl-β-D-galactopyranoside (methyl β-D-labiloside) (**6**). The physical constants of **6** are in good agreement with those quoted by Akita, *et al.*,[6] for methyl β-D-labiloside.

CHART I



1 R = H, R' = SO_2CH_3 **5** R = I, R' = SO_2CH_3

2 R = CH_3, R' = SO_2CH_3 **6** R = R' = H

3 R = CH_3, R' = H

Hydrolysis of the glycoside with sulfuric acid gave 6-deoxy-2,4-di-*O*-methyl-D-galactose (labilose) (**7**) as a white crystalline product with mp 131–134° and $[\alpha]_D$ +86° (water). The recorded values for natural labilose are mp 129°, $[\alpha]^{27}_D$ +82° (water).[6] The enantiomorph of labilose, 2,4-di-*O*-methyl-L-fucose, has been synthesized by Gardiner and Percival[15] and had mp 131–132°, $[\alpha]^{18}_D$ −85° (water).

The lithium aluminum hydride reduction of **2** in ether–benzene gave **6** directly in 60% yield. This difference in product owing to change in solvent, has been noted previously for reductions of other carbohydrate derivatives.[16]

(1) Pioneering Research Division Postdoctoral Research Fellow, 1963–1965.
(2) H. Umezawa and E. Wada, Japanese Patent 8119 (April 24, 1965).
(3) M. Ishizuka, T. Takeuchi, K. Nitta, G. Koyama, M. Hori, and H. Umezawa, *J. Antibiotics* (Tokyo), **17**, 124 (1964).
(4) E. Akita, K. Maeda, and H. Umezawa, *ibid.*, **16**, 147 (1963).
(5) E. Akita, K. Maeda, and H. Umezawa, *ibid.*, **17**, 200 (1964).
(6) E. Akita, K. Maeda, and H. Umezawa, *ibid.*, **17**, 37 (1964).
(7) R. C. Chalk, D. H. Ball, and L. Long, Jr., *J. Org. Chem.*, **31**, 1509 (1966).
(8) R. W. Jeanloz, *J. Am. Chem. Soc.*, **76**, 5684 (1954).
(9) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 506 (1946).
(10) F. Smith, *ibid.*, 1724 (1939).
(11) A. K. Bhattacharyya and H. K. Mukherjee, *Bull. Chem. Soc. Japan*, **37**, 1425 (1964).
(12) A. K. Mitra, D. H. Ball, and L. Long, Jr., *J. Org. Chem.*, **27**, 160 (1962).
(13) S. C. Williams and J. K. N. Jones, *Can. J. Chem.*, **43**, 3440 (1965).
(14) J. M. Sugihara and W. J. Teerlink, *J. Org. Chem.*, **29**, 550 (1964).
(15) J. G. Gardiner and E. Percival, *J. Chem. Soc.*, 1414 (1958).
(16) F. W. Parrish and J. H. Westwood, and L. Long, Jr., in preparation.