General Procedure for UV and Visible Spectra of Azulenium Cations and Charge-Transfer Complexes. A stock solution of the azulene was prepared in $CHCl_3$ at two concentrations, about 10^{-3} M for visible and 10^{-5} M for UV. Solutions of various concentrations of trifluoroacetic acid were prepared in $CHCl_3$. The azulene stock solution (0.5 mL) and trifluoroacetic acid solution (0.5 mL) were mixed in a 1-cm cell of 1-mL volume. Spectra of **16** and **1** with TNB and TCNE were obtained by mixing appropriate concentrations of donor and acceptor in $CHCl_3$ solution and recording the spectrum. No attempts were made to isolate the complexes because of the limited amount of starting materials available.

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Studies on the Syntheses of Heterocyclic Compounds. 700.[1a] Syntheses of Isoquinoline Alkaloids with Cuprous Chloride and Oxygen in Pyridine as an Enzymic Model

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Abstract: Phenol oxidation of (+)-reticuline (**1**) perchlorate with cuprous chloride and oxygen in pyridine gave (+)-corytuberine (**2**), (+)-isoboldine (**3**), and pallidine (**4**). Racemic 1,2,3,4-tetrahydro-7-hydroxy-1-(3-hydroxy-4-methoxyphenethyl)-6-methoxy-2-methylisoquinoline (**5**) hydrochloride yielded the ortho-ortho, ortho-para, and para-para coupling products (racemates of **6**, **7**, and **8**) under the same conditions. (±)-Orientalinone (**10** and/or **11**) and (±)-kreysiginone (**13**) were also synthesized from the corresponding 1-benzyl- and 1-phenethylisoquinolines (**9** and **12**). Oxidation with cupric chloride and potassium superoxide in pyridine also gave rise to similar results. Mechanisms of the oxidations by these reagents are discussed.

The oxidation and coupling of phenols is a subject of great importance in biochemistry and organic chemistry.[2] Furthermore, the reactions between atmospheric oxygen and phenols are of special interest in relation to autoxidation processes and enzymic processes. Oxidative reactions of phenols with molecular oxygen activated by metal salts are well known.[2-8] Many phenolic compounds in nature would also be oxidized with enzymes involving both metal and oxygen to afford complex natural products. The three main classes of enzymes known as catalyst for phenol oxidation and coupling are the laccase, the tyrosinase, and the peroxidase. The former two enzymes include copper ion and oxygen.[2]

In an isoquinoline alkaloid field, biosynthetic pathways to a variety of the alkaloid groups, for example, aporphines, morphines, proaporphines, cularines, and bisbenzylisoquinolines,[9,10] involve phenol oxidative coupling as a key reaction. However, success in demonstrating the coupling with enzymes in vitro has so far been limited.[11-13] There are nu-

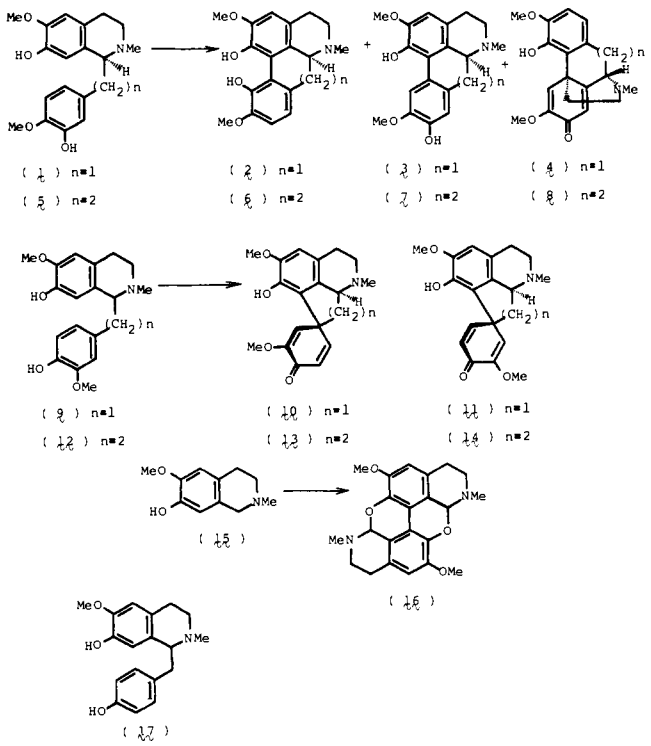
merous examples of phenol oxidation using potassium ferricyanide,[14-20] ferric chloride,[19] vanadium oxychloride,[18,21] thallium tris(trifluoroacetate),[22] and so on as chemical oxidizing agents, although most of them gave unsatisfactory yields. In connection with our interest in alkaloid synthesis under mild conditions[23,24] we studied phenol oxidation of some isoquinoline alkaloids by a mixture of cuprous chloride and molecular oxygen in pyridine[3,25,26] and the related reaction systems, and here wish to report syntheses of several alkaloids, (+)-corytuberine (**2**), (+)-isoboldine (**3**), pallidine (**4**), (±)-orientalinone (**10**), and (±)-kreysiginone (**13**).

Results and Discussion

The suspension of cuprous chloride, Cu_2Cl_2, in pyridine absorbs oxygen rapidly under an oxygen atmosphere to give a dark green solution,[3,25,26] to which a solution of 1 molar equiv of (+)-reticuline (**1**)[27] perchlorate in pyridine was added dropwise at room temperature with efficient stirring. The color of solution changed soon to dark brown. The mixture was further stirred for 15–30 min and crystalline ammonium chloride was then added to the mixture, which was partitioned between chloroform and dilute ammonia. The chloroform extract was purified by preparative TLC on silica gel to afford (+)-corytuberine (**2**)[28,29] (28%), (+)-isoboldine (**3**)[30] (8%), and pallidine (**4**)[30] (6%). (±)-1,2,3,4-Tetrahydro-7-hydroxy-1-(3-hydroxy-4-methoxyphenethyl)-6-methoxy-2-methylisoquinoline (**5**)[31] hydrochloride gave also ortho-ortho (racemate of **6**, 5%), ortho-para (racemate of **7**,[19] 2%), and para-para (racemate of **8**,[31] 2%) coupling products. All products except **6** were identified by direct comparisons with authentic samples. The structure of **6**, which was a very polar compound, was determined from the UV λ_{max} (MeOH) 258 and 295 nm, and mass spectrum showing a strong peak at *m/e* 324 ($M^+ - 17$), which were characteristic for the 1,12-dihydroxyhomoaporphines.[32]

A similar reaction of (±)-orientaline (**9**)[33] perchlorate gave (±)-orientalinone (racemate of **10** and/or **11**)[33,34] (19.4%) and (±)-isoorientalinone (racemate of **10** and/or **11**) (6.5%), while the racemic phenethylisoquinoline (**12**)[19,20] hydrochloride provided (±)-kreysiginone (racemate of **13**)[19,20] (11.4%) and its diastereoisomer (racemate of **14**)[19,20] (26.6%) under similar conditions as above.

Scheme I



(±)-Isoorientalinone (racemate of **11** and/or **10**) had not been previously obtained in the pure state from the epimeric mixture,[33] but, this time, (±)-isoorientalinone was separated from the epimer (racemate of **10** and/or **11**) using high-pressure liquid chromatography. The olefinic β proton adjacent to the methoxy group of (±)-isoorientalinone appeared in the NMR at slightly higher field, δ 5.80 (doublet, J = 2.5 Hz), than that of (±)-orientalinone, δ 5.93 ppm. All chemical shifts of (±)-isoorientalinone except this signal were observed at very similar field to those of (±)-orientalinone.[34]

Corypalline (**15**) was oxidized with 1 molar equiv of cuprous chloride and oxygen in pyridine and methanol to give the diether (**16**) in 38% yield, which is the result of ortho-ortho phenol oxidative coupling followed by oxidation at C-1 of the tetrahydroisoquinoline. The diether (**16**) had been previously obtained from oxidation of *N*-methylcoclaurine (**17**) with potassium ferricyanide.[35]

It is interesting that oxidation of *N*-methylcoclaurine with oxygen–Cu_2Cl_2–pyridine yielded no corresponding proaporphine but rather tarry, unclarified products. One of the latter appeared to be a dimer of *N*-methylcoclaurine because after methylation with diazomethane, the mass spectrum of the crude product showed a peak at *m/e* 650. The product could not be further purified. It is thus probable that the *o*-methoxyphenol moiety is necessary for oxidative coupling in this system.

The ortho-ortho oxidative coupling of reticuline (**1**) to corytuberine (**2**) and of the corresponding phenethylisoquinoline (**5**) to **6** with chemical reagents is the first example.[36]

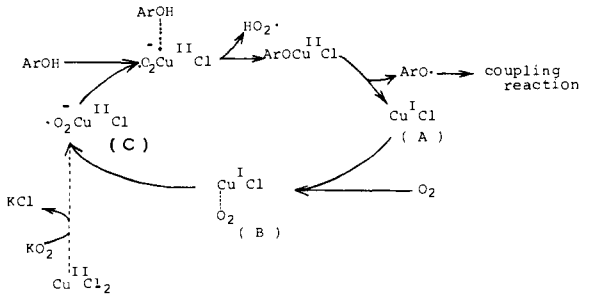
The use of dimethylformamide in place of pyridine for the above reaction gave poor results and needed longer reaction time. Cuprous bromide gave a similar result to cuprous chloride, but the use of divalent copper salts such as cupric chloride instead of cuprous chloride gave no oxidized product.

Two mechanisms could be considered for the above oxidation. The first one resembles that of tyrosinase,[1,37] in which the copper would be in the cuprous state throughout the reaction and the phenols are oxidized directly with activated molecular oxygen to aryloxy radicals as in the following equation.

$$ArOH + O_2 \rightarrow ArO\cdot + HO_2\cdot$$

The second one is relevant to that of laccase[1,38] in which one electron of the phenol group is transferred to molecular oxygen via copper, the valence of which changes during the reaction as shown in Scheme II.

Scheme II



In order to clarify the mechanism of the above oxidation, the following reactions were studied. When a mixture of cupric chloride and an excess of potassium superoxide in pyridine was stirred for 12 h at room temperature under nitrogen atmosphere, the color of the solution changed to dark green. With this mixture, (+)-reticuline (**1**) perchlorate was converted to (+)-corytuberine (**2**) (32.5%), (+)-isoboldine (**3**) (8.5%), and pallidine (**4**) (6.5%), whereas (±)-orientaline (**9**) perchlorate was transformed to a mixture of (±)-orientalinone and its

epimer (**10** and **11**) (25% yield), the ratio of the two components of which was nearly the same as in the case of oxygen–Cu_2Cl_2–pyridine, indicating that a cupric complex such as C in Scheme II would operate in the above oxidative coupling.[39,40] Neither potassium superoxide in pyridine nor potassium superoxide and cuprous chloride in pyridine under a nitrogen atmosphere oxidized the above substrates, but the starting material was recovered.

Furthermore, on oxidation with two 2 molar equiv of a divalent copper complex, $[pyridineCuCl(OMe)]_2$, prepared from cuprous chloride in pyridine in the presence of oxygen and methanol,[7] in pyridine under nitrogen atmosphere, (+)-reticuline (**1**) and (±)-orientaline (**9**) perchlorates afforded the same reaction products as above. It is thus probable that cuprous salts are readily oxidized with molecular oxygen in pyridine to give certain cupric ions, which are efficient catalytic systems for phenol oxidative coupling of some phenolic isoquinoline alkaloids.

Experimental Section

All melting points are uncorrected. UV spectra were taken with a Hitachi 124 spectrometer, IR spectra with a Hitachi 215 spectrometer, NMR spectra with a JNM-PMX-60 spectrometer (tetramethylsilane as an internal reference), and mass spectra with a Hitachi RMU-7 spectrometer. High-pressure liquid chromatographies were carried out with a Waters Associates ALC/GDC 202/R 401 instrument equipped with a column (1 ft × 0.25 in.) packed with μ-Bondapak-C_{18}. Preparative TLC was carried out using Kieselgel HF_{254} (Merck) in chloroform–methanol solvent systems.

Oxidation of (+)-Reticuline (1) Perchlorate. A. With O_2–Cu_2Cl_2–Pyridine. Cuprous chloride (60 mg, 0.3 mmol) was added in portions to dry pyridine (6 mL) under stirring and nitrogen atmosphere at room temperature before the atmosphere was replaced with oxygen. After further stirring for 10 min, a solution of (+)-reticuline (**1**) perchlorate (126 mg, 0.3 mmol) in pyridine (6 mL) was slowly added to the resulting dark green solution under efficient stirring and oxygen atmosphere. The mixture was further stirred for 15–30 min under oxygen atmosphere. Excess of crystalline ammonium chloride was added to the mixture, which was partitioned between 10% ammonia and chloroform. The aqueous solution was further extracted twice with chloroform. The combined chloroform layers were washed with a saturated aqueous ammonium chloride solution, dried over sodium sulfate, and evaporated to give a gum, which was purified by preparative TLC to afford (+)-corytuberine (**2**, 27.4 mg, 28%), mp 238–239 °C (from ethanol) (lit.[29] 238–239 °C), $[\alpha]_D^{20}$ +280° (ethanol), (+)-isoboldine (**3**, 7.8 mg, 8%), mp 123–124 °C (from ethanol) (lit.[30] 123–124 °C), $[\alpha]_D^{20}$ +62° (chloroform), and pallidine (**4**, 7.7 mg, 6%), $[\alpha]_D^{20}$ −30° (ethanol), as a yellow syrup.[30]

B. With KO_2–$CuCl_2$–Pyridine. A mixture of cupric chloride (71.2 mg, 0.52 mmol) and potassium superoxide (83.6 mg, 1.56 mmol) in pyridine (6 mL) was stirred for 12 h at room temperature under nitrogen. After the addition of (+)-reticuline (**1**) perchlorate (109 mg, 0.26 mmol) in pyridine (3 mL) to the above mixture, the reaction mixture was stirred for 1.5 h at room temperature under nitrogen. Workup as above gave (+)-corytuberine (**2**, 27 mg, 32.5%), (+)-isoboldine (**3**, 7.2 mg, 8.5%), and pallidine (5.5 mg, 6.5%).

Oxidation of (±)-Orientaline (9) Perchlorate. A. With O_2–Cu_2Cl_2–Pyridine. (±)-Orientaline (**9**) perchlorate (86 mg, 0.2 mmol) in pyridine (2 mL) was treated as in A with cuprous chloride (40 mg, 0.2 mmol) in pyridine (2 mL). Workup as above followed by purification by preparative TLC gave an epimeric mixture, which was further purified by high-pressure liquid chromatography. Elution was carried out with methanol–water containing 0.5% ammonium carbonate (2:3 v/v) at 3.5 mL min^{-1}. The faster eluate (retention time 6 min) gave (±)-orientalinone (racemate of **10** and/or **11**) (13 mg, 19.4%), mp 233–235 °C dec (from benzene) (lit.[34] mp 233–235 °C dec). The slower eluate (retention time 8 min) gave (±)-isoorientalinone (racemate of **11** and/or **10**) (4 mg, 6.5%): mp 147–149 °C dec (from benzene–hexane); IR ν_{max} ($CHCl_3$) 3530 (OH), 1655, 1635, and 1610 cm^{-1} (dienone system); NMR ($CDCl_3$) δ 2.39 (3 H, s, NMe), 3.64 (3 H, s, OMe), 3.80 (3 H, s, OMe), 5.80 (1 H, d, J = 2.5 Hz, olefinic β proton adjacent to methoxy group), 6.38 (1 H, J = 9.5 Hz, olefinic α proton), 6.58 (1 H, s, ArH), and 6.85 (1 H, dd, J = 2.5 and 9.5 Hz, olefinic β proton); m/e 327 (M^+).

B. With KO_2–$CuCl_2$–Pyridine. (±)-Orientaline (**9**, 21.5 mg, 0.05 mmol) in pyridine (1 mL) was treated as in B with cupric chloride (13.5 mg, 0.1 mmol) and potassium superoxide (22 mg, 0.3 mmol). Workup followed by purification by preparative TLC gave an epimeric mixture (4 mg, 25%) of (±)-orientalinone and (±)-isoorientalinone (3:1).

Oxidation of (±)-1,2,3,4-Tetrahydro-7-hydroxy-(3-hydroxy-4-methoxyphenethyl)-6-methoxy-2-methylisoquinoline (5). The phenethylisoquinoline (**5**) hydrochloride (76 mg, 0.2 mmol) in pyridine (3 mL) was treated as in the case of the oxidation of (+)-reticuline by method A, with cuprous chloride (40 mg, 0.2 mmol) in pyridine (3 mL). Workup followed by purification by preparative TLC gave the homomorphinandienone (**8**, 1.3 mg, 2%), mp 220–221 °C (from chloroform–benzene) (lit.[31] mp 220–221 °C), the 1,10-dihydroxyhomoaporphine (**7**, 1.3 mg, 2%), mp 241–242 °C (from methanol (lit.[19] mp 241–242 °C), and the 1,12-dihydroxyhomoaporphine (**6**, 3.4 mg, 5%) as a powder: mp 210–217 °C (from chloroform–benzene); UV λ_{max} (MeOH) 258 and 295 nm; IR ν_{max} ($CHCl_3$) 3550 cm^{-1} (OH); m/e 324 ($M^+ - 17$).

Oxidation of (±)-1,2,3,4-Tetrahydro-7-hydroxy-1-(4-hydroxy-3-methoxyphenethyl)-6-methoxy-2-methylisoquinoline (12). The phenethylisoquinoline (**12**) hydrochloride (380 mg, 1 mmol) in pyridine (10 mL) was treated, in the same manner as the oxidation of (+)-reticuline by method A, with cuprous chloride (200 mg, 1 mmol) in pyridine (10 mL). After evaporation of the pyridine under 40 °C, an excess of crystalline ammonium chloride was added to the residue, which was partitioned between chloroform and 10% ammonia. The chloroform layer was washed with saturated aqueous ammonium chloride, dried over sodium sulfate, and evaporated to give a gum (330 mg), which was chromatographed on silica gel. Elution with chloroform–methanol (99:1 v/v) gave an epimeric mixture (150 mg) of the dienones. Fractional crystallization from benzene gave (±)-kreysiginone (racemate of **13**) (39 mg, 11.4%), mp 145–149 °C (from benzene) (lit.[19] 145–149 °C), and the diastereoisomer (racemate of **14**) (90.1 mg, 26.6%), mp 190–193 °C (from benzene–hexane) (lit.[19] 190–193 °C).

Oxidation of Corypalline (15). A suspension of cuprous chloride (91 mg, 0.46 mmol) in pyridine (10 mL) and methanol (0.5 mL) was stirred for 1 h at room temperature under oxygen atmosphere. A solution of corypalline (**15**, 80 mg, 0.42 mmol) in pyridine (5 mL) was slowly added to the above mixture and the resulting mixture was stirred for 1 h under oxygen. After the addition of crystalline ammonium chloride, the mixture was partitioned between chloroform and 10% ammonia. The aqueous layer was extracted with chloroform. The combined chloroform layers were washed with water, dried over sodium sulfate, and evaporated to give a brown solid, which was recrystallized from ethanol–ether to afford the diether **16** (30 mg, 38%), mp 218–220 °C (lit.[35] 219–220 °C).

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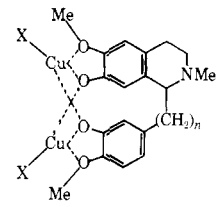

(37) D. Kertesz and R. Zito, "Oxygenases", O. Hayaishi, Ed., Academic Press, New York, N.Y., 1962, p 317.
(38) P. D. McDonald and G. A. Hamilton, "Oxidation in Organic Chemistry", Part B, W. S. Trahanovsky, Ed., Academic Press, New York, N.Y., 1973, pp 123–133.
(39) The above phenol oxidation with oxygen–Cu_2Cl_2–pyridine or KO_2–$CuCl_2$–pyridine could not be inhibited in the presence of 5×10^{-6} M bovine erythrocyte superoxide dismutase (SOD) (Sigma).
(40) There is another possibility that cupric chloride could be reduced with potassium superoxide to give cuprous salt and molecular oxygen as follows:[41] $CuCl_2 + KO_2 \rightarrow CuCl + O_2 + KCl$.
(41) J. S. Valentine and A. B. Curtis, *J. Am. Chem. Soc.*, **97**, 224 (1975).

Isomerization of (*Z*)-Arenediazo Thioethers on Aldolase and Model Compounds

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Abstract: Arenediazo derivatives of *N*-acetylcysteine, having different substituent groups in para position, have been prepared. The decomposition and isomerization kinetics of the *Z* isomers of these chromophores have been investigated in water solution. The nature of the para substituent group in the aryl moiety affects activation parameters for both reactions. Electron-withdrawing groups stabilize the *Z* isomer giving slower reaction rates for both thermal conversion to the *E* isomer and decomposition; electron-donating groups bring about rate enhancements. A linear enthalpy–entropy relationship and a fair linearity in the Hammett plot support the existence of a unique isomerization mechanism. The *p*-nitro- and *p*-carboxy-substituted benzenediazo thioethers have been formed on aldolase. In both cases the protein moiety lowers the activation energy of the isomerization. With the *p*-carboxybenzeneazoaldolase a first-order decay of the *Z* isomer is observed when the number of extrinsic chromophores is one per protein chain; when the number is two (in this case they are known to be located at Cys 237 and Cys 287), the time decay curve can be produced by the sum of two different exponential components, one of which is similar to that observed when only one extrinsic chromophore is present per chain. The two processes have been rationalized tentatively with the different location of the two chromophores on the enzyme.

Diazo thioether chromophores are formed by coupling diazonium salts with proteins having free sulfhydryl groups.[2a] They are fairly stable in neutral solutions, and show photosensitivity due to *E*/*Z* isomerization.[2b] A biologically active azoaldolase has been obtained, in which the modified cysteine residues are Cys 237 and Cys 287.[3] This provides the possibility of using the diazo thioethers as optical probes[4] for selected regions of a protein.

The properties of these chromophores are markedly influenced by the substituent in the para position of the aryl moiety. In order to choose the most suitable group for a protein probe, as regards chemical stability, lifetime of the metastable *Z* state, and sensitivity to the environment, we have studied a series of compounds, prepared by reaction of *N*-acetyl-L-cysteine with the aromatic diazonium salts, bearing the appropriate substituent in the para position of the aryl moiety (Scheme I).

The p-NO_2 and p-COO^- substituted benzenediazo thioethers have been investigated covalently linked to aldolase. Indications of interaction with the protein have been found. The properties of the *p*-carboxylate benzenediazo chromophore suggest its possible application as an optical probe for local protein conformation.

Experimental Section

Cysteine Derivatives. Benzenediazo-substituted cysteine derivatives were prepared as already reported[5] by coupling the appropriate diazonium salt with a water solution of *N*-acetyl-L-cysteine (Serva)