Tetrahedron Vol. 42, No 5, pp. 1475 to 1485, 1986
Printed in Great Britain.

0040-4020/86 $3.00 + .00
© 1986 Pergamon Press Ltd.

TOTAL SYNTHESIS OF ORELLANINE

THE LETHAL TOXIN OF *CORTINARIUS ORELLANUS* FRIES MUSHROOM[1]

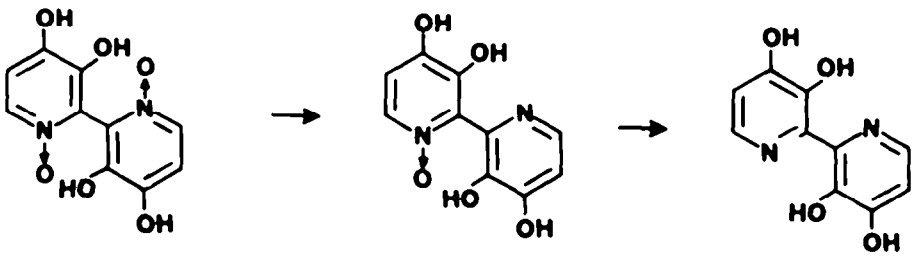
MARCELLO TIECCO,*[a] MARCO TINGOLI,[a] LORENZO TESTAFERRI,[a] DONATELLA CHIANELLI,[a] and ERNEST WENKERT.*[b]

a) Istituto di Chimica Organica, Facoltà di Farmacia, Università di Perugia, Italy.
b) Department of Chemistry, University of California - San Diego, La Jolla, California 92093.

(*Received in UK 6 November* 1985)

Abstract. The syntheses of the main toxin of *Cortinarius Orellanus* Fries, orellanine, and of its decomposition product, orelline, are reported. The structures of 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl-N,N'-dioxide for orellanine and of 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl for orelline have been proposed by W. Z. Antkowiak and W. P. Gessner. These two compounds have now been synthesized starting from the 3,3',4,4'-tetramethoxy-2,2'-bipyridyl which could be obtained in good yields by the nickel-phosphine complex-mediated homo coupling of 2-bromo-3,4-dimethoxy pyridine according to the general procedure previously reported by us. The 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl was obtained by demethylation with hydrobromic acid. The oxidation of this compound with hydrogen peroxide afforded the 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl-N,N'-dioxide. This latter compound was also obtained by the demethylation of the 3,3',4,4'-tetramethoxy-2,2'-bipyridyl-N,N'-dioxide with hydrobromic acid. The products obtained presented physical and spectral properties identical to those of the natural products.

The toxic properties of the mushroom *Cortinarius Orellanus* Fries were discovered in 1952 after the massive fatal poisoning which occurred in the Konin district in Poland. From the methanolic



1 **Orellanine**

2 **Orellinine**

3 **Orelline**

extract of these fungi Grzymala[2] was able to isolate a white crystalline substance which was named orellanine. Orellanine was considered to be the toxin of the *Cortinarius Orellanus* since it presented toxic effects identical to those caused by the intact fungi. Grzymala found that orellanine slowly decomposes when heated above 150°C and gives explosive decomposition when heated above 267°C. The product of this decomposition was a yellow non toxic sublimable compound. Antkowiak and Gessner, using an improved isolation procedure, were able to isolate orellanine and its decomposition product in a pure form and described their IR and UV spectra.[3] After careful chemical and spectral investigations these authors proposed for orellanine the structure of 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl-N,N'-dioxide (1) and for its yellow decomposition product, which they called orelline, the structure of 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl (3).[4] Orelline was also detected in the methanolic extract of the mushroom.

Very recently Antkowiak and Gessner[5] studied the chemical transformations of orellanine and found that on heating above 180°C or by reaction with hydrogen in the presence of Pt or by UV irradiation orellanine is transformed into the mono N-oxide and into orelline by stepwise loss of the two oxygen atoms of the N-oxide functions. The mono N-oxide, which was called orellinine (2), was found to have a toxicity similar to that of orellanine. All these three substances were found to be present in the methanolic extract of *Cortinarius Orellanus* although orellanine was by far the most abundant component.[5] Orellanine, orellinine and orelline were found to be present also in the methanolic extracts of *Cortinarius Speciosissimus* mushroom.[5,6] The facile stepwise oxygen elimination from the molecule of orellanine (during pyrolysis as well as during mass fragmentation) is peculiar since the N-oxide function in bipyridyl derivatives generally demonstrates a great thermal stability.[7] Using the 2-(2'-hydroxyphenyl) pyridine N-oxide as a model compound, Antkowiak and Gessner suggested that the thermal deoxidation might occur through a [1,5] oxygen shift from the nitrogen atom to the oxygen atom of the hydroxy group in the 3'-position to give an hydroperoxide which easily decomposes.[8]

In order to have an independent evidence which could firmly establish the interesting and peculiar structures proposed for the natural products, it seemed desirable to carry out an unambiguous synthesis of orellanine and orelline. Although this problem can be approached in several ways we thought that the easiest way to obtain the desired compounds was to dispose of the 3,3',4,4'-tetramethoxy-2,2'-bipyridyl (tetramethyl orelline). This compound in fact could be demethylated to orelline and orellanine could be then obtained by oxidation; otherwise, tetramethyl orelline could be transformed into its N,N'-dioxide which could be demethylated to orellanine from which orelline could be obtained by deoxidation. The success of these processes thus requires a good synthetic method for the preparation of the key intermediate, 3,3',4,4'-tetramethoxy-2,2'-bipyridyl. Preliminary experiments, using the methods described in the literature for the synthesis of bipyridyls, were rather discouraging since the desired product could be obtained in very poor yields. Our previous experience in the reactions catalyzed by low-valent nickel complexes induced us to attempt the homo coupling of the 2-bromo-3,4-dimethoxy pyridine under the influence of nickel-triphenylphosphine complexes. This reaction was successful and we found that it could be applied to the synthesis of other bipyridyl derivatives as well as to other biaryl compounds. Several examples of the utility of this very simple and powerful method have been reported in our previous paper.[9] In this way the key intermediate, 3,3',4,4'-tetramethoxy-2,2'-bipyridyl was easily available and the syntheses of orellanine and orelline could be successfully carried out according to both the reaction sequences indicated above. The various steps are summarized in Schemes 1 and 2 and discussed under the Results and

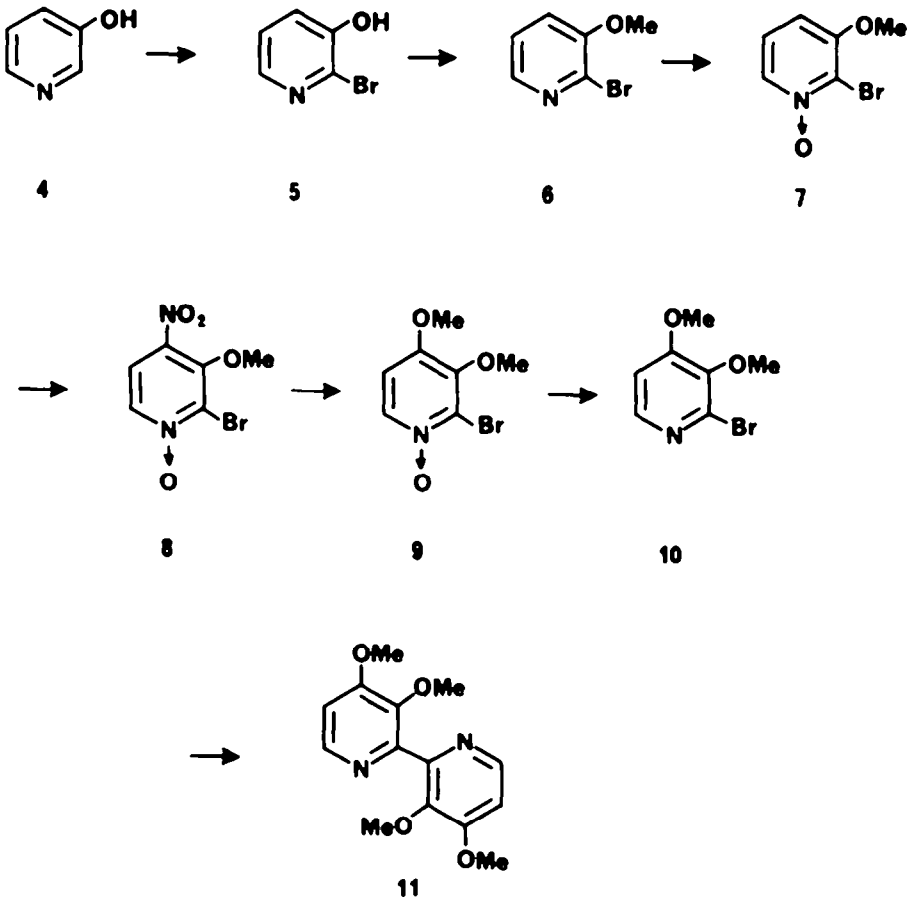
Discussion Section.

While this paper was in an advanced stage of preparation Dehmlow and Schulz reported a synthesis of orellanine and orelline.[10] The synthetic strategy employed by these authors is substantially identical to one of the two methods reported in this paper and the 3,3',4,4'-tetramethoxy-2,2'-bipyridyl was prepared from the 2-chloro-3,4-dimethoxy pyridine using the homo coupling procedure described by us.[9]

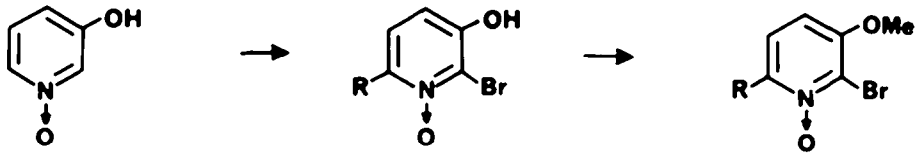
RESULTS AND DISCUSSION

The reactions employed for the synthesis of the 3,3',4,4'-tetramethoxy-2,2'-bipyridyl (11) are indicated in Scheme 1. Commercially available 3-hydroxy pyridine (4) was treated with bromine in

SCHEME 1



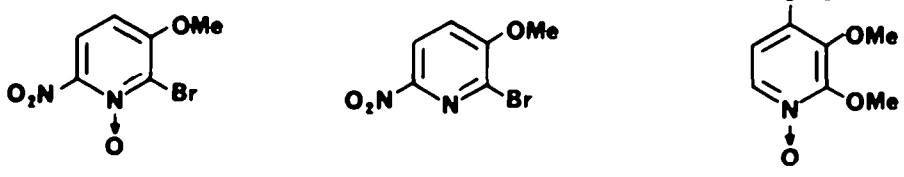
alkaline solution[11] to afford the 2-bromo derivative (5) in 30 - 35% yields. O-Alkylation of the 2-bromo,3-hydroxy pyridine (5) to the 2-bromo,3-methoxy pyridine (6) could be effected in high yields working in DMF.[12] Oxidation with *m*-chloroperbenzoic acid in chloroform afforded the 2-bromo,3-methoxy pyridine N-oxide (7) in 85 - 90% yields. Compound (7) could also be obtained starting from the commercially available 3-hydroxy pyridine N-oxide (12) by reaction with bromine in alkaline solution [11] followed by alkylation of the 2-bromo,3-hydroxy pyridine N-oxide (13) thus



12 13 : R = H 7 : R = H

14 : R = Br 15 : R = Br

obtained. Compound (13) was formed in good yields (60 - 70%) but it was contaminated by small amounts of the 2,6-dibromo derivative (14) which could not be easily eliminated. Alkylation of (13), under the conditions employed for (5), gave, after column chromatography, the desired compound (7) (40 - 45% yields), the 2,6-dibromo,3-methoxy pyridine N-oxide (15) and a considerable amount (35 - 40%) of unchanged (13). Owing to the complications encountered with this method, it is preferable to synthesize (7) according to the procedure described in Scheme 1. Nitration of (7) with nitric and sulphuric acid afforded an 80 - 85% yields of an almost equimolecular mixture of the 2-bromo,3-methoxy,4-nitro pyridine N-oxide (8) and of the 2-bromo,3-methoxy,6-nitro pyridine N-oxide (16). The two isomers could be easily separated since the 6-nitro derivative (16) was almost completely insoluble when the reaction mixture was poured on water. In the attempt to improve the yields of the 4-nitro derivative we have also carried out the nitration of the 2-bromo,3-methoxy pyridine (6); from this reaction however the only product obtained in 80 % yield was the 2-bromo,3-methoxy,6-nitro pyridine (17).



16 17 18

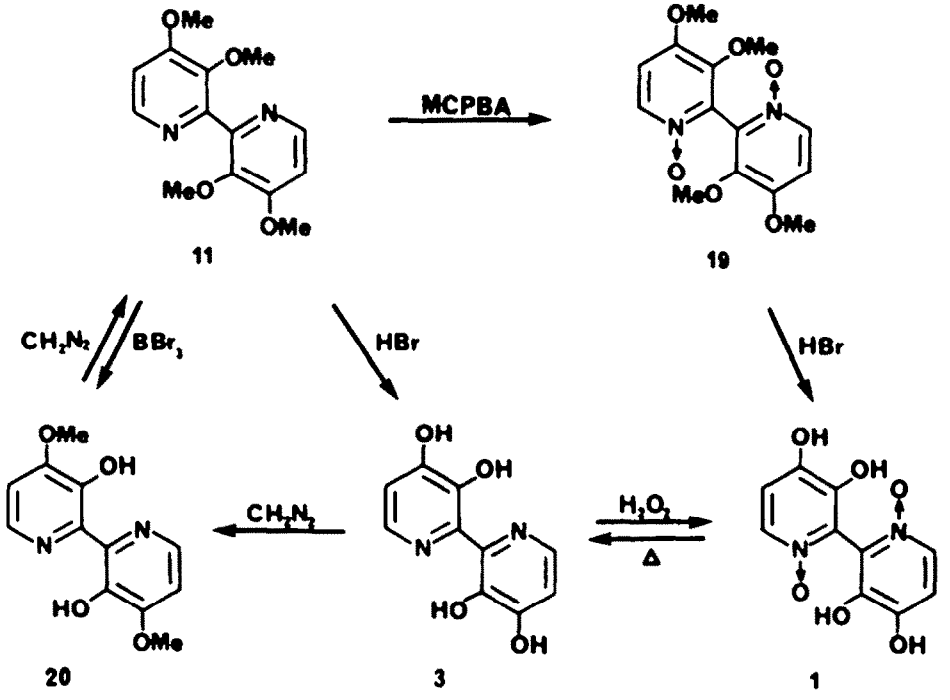
Substitution of the nitro group in (8) by the methoxy was effected with sodium methoxide in methanol. Care must be taken in this reaction (see Experimental) since substitution can also involve the bromine atom and some of the desired 2-bromo-3,4-dimethoxy pyridine (9) can be transformed into the 2,3,4-trimethoxy pyridine (18). Finally deoxygenation of (9) to the 2-bromo-3,4-dimethoxy pyridine (10) was carried out with phosphorus tribromide; in this way compound (10) was obtained in 80 - 85% yields. The use of PCl_3 resulted in partial exchange of the bromine atom with chlorine and was therefore avoided.

The coupling of (10) to obtain the tetramethyl orelline (11) was first attempted with copper in DMF at 150° but the yields were extremely poor (less than 10%). Then we used the recently described method which utilizes palladium on charcoal under phase-transfer conditions[13] but the results obtained were still very unsatisfactory, (11) being formed in only 15 - 20% yields. At

this point we looked for a new method and this led us to the discovery of the very efficient and extremely versatile homo coupling of halopyridines and other haloaromatic compounds mediated by nickel-phosphine complexes which we have published in a previous paper.[9] Application of this method to (10) gave rise to the production of the 3,3',4,4'-tetramethoxy-2,2'-bipyridyl (11) in yields ranging from 75 to 87%.

The bipyridyl (11) was the starting product for the synthesis of orelline (3), orellanine (1) and the other related compounds which are indicated in Scheme 2.

SCHEME 2



Dealkylation of (11) with boron tribromide in refluxing 1,2-dichloroethane[14] gave rise to a product of partial demethylation which was identified as a symmetrical dimethyl orelline on the basis of its proton and carbon-13 nmr spectra. The behaviour of this yellow product towards UV irradiation (see below) suggests that its most probable structure is that of the 3,3'-dihydroxy-4,4'-dimethoxy-2,2'-bipyridyl (20). Dealkylation of (11) with 48% hydrobromic acid[15] at 150°C, in sealed tube, gave rise, after work up, to the desired 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl (orelline) (3) in 50 - 60% yields. This compound was purified by sublimation to give bright yellow crystals. All the physical and spectral data (see Experimental) were identical to those reported by Antkowiak and Gessner[4] for the natural orelline and to those reported by Dehmlow and Schulz[10] for the compound obtained under conditions essentially similar to those described above.

The behaviour of (3) towards diazomethane was investigated following the reactions by tlc on silica gel. Using 2.5 molar equivalents of CH_2N_2, (3) was transformed into the 3,3'-dihydroxy-4,4'-dimethoxy-2,2'-bipyridyl (20). With an excess of CH_2N_2 (20) disappeared and a new spot was revealed on the tlc plate with an R_f identical to that of the tetramethyl orelline (11). The formation of a single tetramethyl derivative from the reaction of the natural orelline

with diazomethane has been reported by Antkowiak and Gessner[4] also, but the only physical property described was the value of the molecular ion (M^+ = 276) and a full comparison with compound (11) was therefore not possible.

The synthesis of the 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl-N,N'-dioxide (orellanine) (1) was easily carried out by the oxidation of orelline (3) with 35% hydrogen peroxide. A 79 - 87% yield of a white crystalline product was obtained. Identification was effected by proton and carbon-13 nmr, mass and UV spectra, as well as by elemental analysis (see Experimental). The behaviour of this compound towards heating and UV irradiation and the solubility tests in various media are described in detail under the Experimental Section. All the physical and spectral data of the synthetic orellanine are identical to those reported for the natural product.[4]

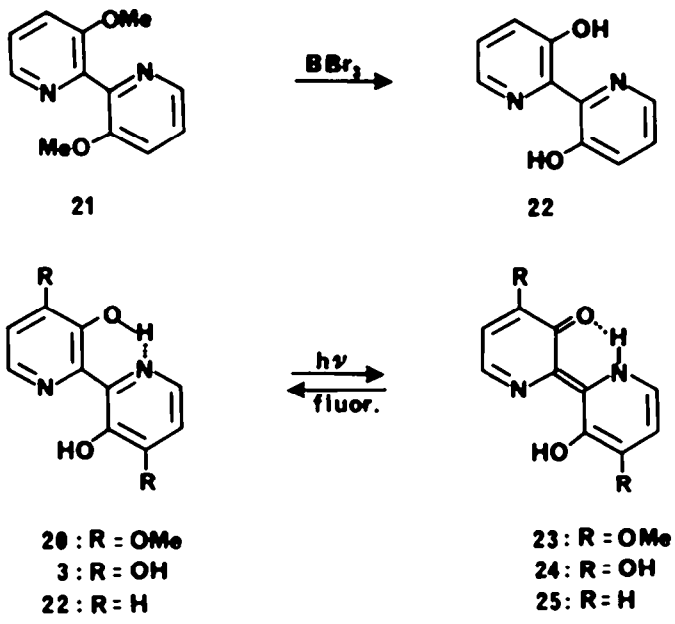
Sublimation of (1), under reduced pressure (0.01 mm/Hg, 220°C), produced the 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl (orelline) (3) in 50% yields.

The synthesis of orellanine was also effected by the dealkylation of the 3,3',4,4'-tetramethoxy-2,2'-bipyridyl-N,N'-dioxide (19). The 3,3',4,4'-tetramethoxy-2,2'-bipyridyl (11) was easily oxidized by excess m-chloro perbenzoic acid, in chloroform, to the corresponding N,N'-dioxide (tetramethyl orellanine) (19) in 75 - 85% yields. The demethylation of (19) was effected with 48% hydrobromic acid, at 120°C for 5 h. Evaporation of the excess acid left a solid residue which was dissolved in water. The pH was adjusted to 5 and a white solid was obtained (54 - 60% yields). The physical and spectral data were identical to those observed for the orellanine obtained by oxidation of orelline, and corresponded to those reported for the natural[4] and the synthetic[10] compound.

As anticipated above the product deriving from incomplete demethylation of (11) is suggested to have the structure of 3,3'-dihydroxy-4,4'-dimethoxy-2,2'-bipyridyl (20). Proton and carbon-13 nmr spectra indicate that this compound is a symmetrical derivative so that the only alternative structure could be that of the 3,3'-dimethoxy-4,4'-dihydroxy-2,2'-bipyridyl. However, this compound, when exposed to UV light, shows a characteristic blue fluorescence, similar to that observed for orelline,[5] and for the 3,3'-dihydroxy-2,2'-bipyridyl (22) which we have obtained in 60% yields from the 3,3'-dimethoxy-2,2'-bipyridyl (21) by dealkylation with BBr_3 (Scheme 3). All these compounds share the common feature of having hydroxy groups in the 3-position of a 2,2'-bipyridyl system. Other products having similar structures are reported to present the same behaviour towards UV irradiation.[16,17] The fluorescent properties presented by these compounds have been interpreted in terms of keto-enol phototautomerism[16] (20) ⇌ (23), (3) ⇌ (24), (22) ⇌ (25) as indicated in Scheme 3. On the basis of these observations we think that the fluorescence presented by the product obtained from the partial demethylation of (11) strongly supports the proposed structure (20). Additional evidences can be obtained from the comparison of the proton chemical shift of the methoxy group in compounds (20), (11) and (21) (see Experimental).

The behaviour of (11) towards the boron tribromide/dimethyl sulphide complex is interesting. This reagent generally gives rise to the dealkylation of all the aryl alkoxy functions which are present in the molecule.[14,15] In the present case the demethylation reaction occurred selectively at the methoxy groups in the 3,3'-positions of the 2,2'-bipyridyl system without affecting those at the 4,4'-positions. Moreover, when (20) was subjected to further reaction with the same reagent it was recovered unchanged. In order to effect the complete demethylation of (11) and of its N,N'-dioxide (19) it was necessary to use the drastic conditions required by the use of hydrobromic acid.

SCHEME 3



In conclusion the total synthesis of the 3,3'4,4'-tetrahydroxy-2,2'-bipyridyl (1), starting from the 3,3'4,4'-tetramethoxy-2,2'-bipyridyl (11), was achieved in two ways: by the oxidation of the 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl (3) and by the demethylation of its tetramethyl derivative (19). Orelline (3) was obtained in two ways also: by the demethylation of (11) and by the deoxidation of (1). The whole of the chemical, physical and spectral data here reported for compounds (3) and (1), as well as those similarily obtained by Dehmlow and Schulz,[10] firmly establish the correctness of the structural attribution given by Antkowiak and Gessner[4] for the naturally occurring orelline and orellanine, respectively.

EXPERIMENTAL

Melting points were taken on a Reichert micro hotstage and are uncorrected. Elemental analyses were carried out on a Carlo Erba Model 1106 Elemental Analyzer. Proton nmr spectra were recorded at 90 MHz on a Varian EM390 instrument in $CDCl_3$ solutions (unless otherwise specified) using TMS as reference. Carbon-13 nmr spectra were recorded at 20.15 MHz on a Bruker WP80SY instrument, operating in the Fourier transform mode with proton decoupling throughout, in $CDCl_3$ solutions (unless otherwise specified) using TMS as reference. Mass spectra were obtained with a Varian MAT 311A instrument at 70 eV using an all glass inlet system. UV spectra were recorded on a Perkin-Elmer Model 551S spectrophotometer. The progress of the reactions described below was monitored by tlc; the experiments were carried out several times and the yields reported represent the range between the minimum and the maximum value obtained.

2-Bromo,3-hydroxy pyridine, (5). A solution of bromine (2 ml) in 10% sodium hydroxide (100 ml) was slowly added (4 - 5 h) to a stirred solution of 3-hydroxy pyridine (4) (10 g) in 10% sodium hydroxide (100 ml). Stirring was continued for 20 - 24 h. The solution was neutralized with conc. hydrochloric acid and the solid formed was filtered off. The product was dried under vacuum. Examination by tlc showed the presence of some unreacted (4). The product was therefore purified by column chromatography on silica gel using a 95 : 5 mixture of chloroform and methanol. Yields ranged from 5.5 to 6.4 g (30 - 35%); m.p. 184-5°C (Lit.[11] 185-6°C, Lit.[18] 186.5-187°C). ^{1}H-nmr (CD_3OD) δ 7.8 - 7.55 (m, 1H), 7.2 - 6.9 (m, 2H), 4.85 (broad s, 1H).

2-Bromo,3-methoxy pyridine, (6). Sodium (0.35 g) was dissolved in methanol (10 ml) and a solution of (5) (2.5 g) in DMF (25 ml) was added. Methanol was distilled off under vacuum (18 mm/Hg) and to the resulting mixture methyl iodide (2.14 g) was added. Stirring was continued at room temperature for 1 - 1.5 h and then the DMF was distilled under vacuum. The residue was taken up with ether and water and the two layers were separated. The organic phase was washed with a saturated sodium chloride solution, dried and evaporated. The product thus obtained (2.10 - 2.30 g; 78 - 85%) did not require further purification. M.p. 43-5°C (Lit.[12] 45°C). ^{1}H-nmr δ 7.9 - 7.75 (m, 1H), 7.2 - 6.9 (m, 2H), 3.85 (s, 3H).

2-Bromo,3-hydroxy pyridine N-oxide, (13). This product was obtained by the bromination of 3-hydroxy pyridine N-oxide (12) (10 g) with the same procedure described above for the preparation of (5). Yields were of the order of 60 - 70%. M.p. 180-5°C (Lit.[11] 178-80°C). ^{1}H-nmr (DMSO-d_6) δ 7.9 (dd, 1H, J = 1.5 and 6.3 Hz), 7.1 (dd, 1H, J = 6.3 and 8.1 Hz), 6.8 (dd, 1H, J = 1.5 and 8.1 Hz), 3.3 (broad s, 1H). The spectrum showed the presence of small amounts of the 2,6-dibromo,3-hydroxy pyridine N-oxide (14).

2-Bromo,3-methoxy pyridine N-oxide, (7). A) A solution of 2-bromo,3-methoxy pyridine (6) (5.8 g) and m-chloroperbenzoic acid (80% pure; 8 g) in chloroform was stirred at room temperature for 2.5 h. The solvent was evaporated at room temperature under vacuum and the residue was chromatographed through an alumina column. Elution with chloroform gave 5.35 - 5.65 g (85 - 90%) of (7). M.p. 159-61°C, ^{1}H-nmr δ 8.05 (dd, 1H, J = 6.3 and 1.2 Hz), 7.2 (dd, 1H, J = 6.3 and 8.4 Hz), 6.8 (dd, 1H, J = 1.2 and 8.4 Hz), 4.0 (s, 3H). ^{13}C-nmr δ 156.4, 133.4, 125.8, 123.0, 108.0, 57.1. Anal. Calcd for $C_6H_6BrNO_2$: C, 35.32; H, 2.97; N, 6.87. Found: C, 35.32; H, 2.87; N, 6.77.
B) Crude (13) (5 g) was methylated according to the procedure described for the preparation of (6). Extractions were effected with chloroform and the residue was chromatographed through a silica gel column using a 92 : 8 mixture of chloroform and methanol. The first fractions contained small amounts of the 2,6-dibromo,3-methoxy pyridine N-oxide (15), m.p. 198-200°C, ^{1}H-nmr δ 7.25 (d, 1H, J = 7.8 Hz), 6.95 (d, 1H, J = 7.8 Hz), 3.85 (s, 3H). Compound (7) was then isolated. Further elution led to the recovery of 30 - 35% of the unreacted starting compound (13).

2-Bromo,3-methoxy,4-nitro pyridine N-oxide (8) and 2-Bromo,3-methoxy,6-nitro pyridine N-oxide, (16). Compound (7) (2.4 g) was dissolved in conc. H_2SO_4 (5.5 ml) and a mixture of conc. H_2SO_4 (5.5 ml) and fuming HNO_3 (7.7 ml) was added. The mixture is immersed into an oil bath kept at 60°C, stirred for 1 h and then poured into ice. The formed precipitate was filtered off and dried under vacuum (0.95 - 1.0 g; 35 - 37%). This product was identified as the 6-nitro derivative (16); m.p. 215-7°C; ^{1}H-nmr (DMSO-d_6) δ 8.05 (d, 1H, J = 8.4 Hz), 7.25 (d, 1H, J = 8.4 Hz), 4.0 (s, 3H); ^{13}C-nmr (DMSO-d_6) δ 119.1, 109.1, 58.0. Anal. Calcd for $C_6H_5BrN_2O_4$: C, 28.94; H, 2.03; N, 11.25. Found: C, 28.85; H, 2.14; N, 11.15. The filtrate was extracted with chloroform for several times, washed with water, dried and evaporated. The residue was chromatographed through a silica gel column using a 80 : 20 mixture of light petroleum and ether. Pure 2-bromo,3-methoxy,4-nitro pyridine N-oxide (8) (1.2 - 1.3 g; 45 - 48%) was obtained. M.p. 113-5°C; ^{1}H-nmr δ 8.1 (d, 1H, J = 7.0 Hz), 7.75 (d, 1H, J = 7.0 Hz), 4.05 (s, 3H). ^{13}C-nmr δ 136.2, 119.0, 62.9. Anal. Found: C, 29.00; H, 2.11; N, 11.05.

2-Bromo,3-methoxy,6-nitro pyridine, (17). The 2-bromo,3-methoxy pyridine (6) (1 g) was nitrated under the conditions described above for the preparation of compound (8). The mixture was poured into ice and made alkaline by addition of ammonia. Extraction with chloroform gave, after the usual work up, a residue which was chromatographed through a silica gel column using a 90 : 10 mixture of light petroleum and ether. Pure (17) was thus obtained (1 g; 80%), m.p. 140-2°C, ^{1}H-nmr δ 8.25 (d, 1H, J = 8.4 Hz), 7.35 (d, 1H, J = 8.4 Hz), 4.05 (s, 3H); ^{13}C-nmr δ 131.0, 119.3, 119.1, 57.4. Anal. Calcd for $C_6H_5BrN_2O_3$: C, 30.92; H, 2.17; N, 12.02. Found: C, 30.95; H, 2.10; N, 12.24.

2-Bromo-3,4-dimethoxy pyridine N-oxide, (9). Sodium (0.1 g) is dissolved in methanol and compound (8) (0.6 g) was added. The mixture is stirred at room temperature and the progress of the reaction is monitored by tlc. After 1.5 - 2 h the starting product has been consumed and product (9) is formed together with small amount of another compound. The amount of this undesired product rapidly increases at the expenses of (9) if the methoxide is not destroyed. For this purpose the reaction mixture is passed through the ion-exchange resin Amberlite IR-120, which had been washed with 5% hydrochloric acid, water and methanol. The methanol was evaporated and the residue was chromatographed through a silica gel column using a 97 : 3 mixture of chloroform and methanol to afford pure (9) (0.41 - 0.44 g; 72 - 78%) m.p. 97-8°C; ^{1}H-nmr δ 8.2 (d, 1H, J = 7.0 Hz), 6.95 (d, 1H, J = 7.0 Hz), 4.0 (s, 3H), 3.9 (s, 3H); ^{13}C-nmr δ 151.4, 146.2, 135.7, 131.8, 107.5, 61.0, 56.7. Anal. Calcd for $C_7H_8BrNO_3$: C, 35.92; H, 3.45; N, 5.99. Found: C, 36.05; H, 3.27; N, 5.89.

Further elution afforded small amounts of an oil which was identified as the 2,3,4-trimethoxy pyridine N-oxide (18); ^{1}H-nmr δ 8.0 (d, 1H, J = 7.0 Hz), 6.75 (d, 1H, J = 7.0 Hz), 4.2 (s, 3H), 3.97 (s, 3H), 3.92 (s, 3H). ^{13}C-nmr δ 152.9, 138.4, 134.4, 107.3, 103.2, 61.2, 60.4, 56.2. Anal. Calcd for $C_8H_{11}NO_4$: C, 51.88; H, 6.00; N, 7.56. Found: C, 51.91; H, 5.89; N, 7.48.

2-Bromo-3,4-dimethoxy pyridine, (10). To a solution of the N-oxide (6) (2.5 g) in $CHCl_3$ (60 ml), stirred at 60°C, phosphorus tribromide was added dropwise in few minutes. After half an hour analysis of the reaction mixture by tlc showed that the transformation was complete. The cooled reaction mixture is treated with dilute NaOH solution and the two layers were separated. The chloroform was washed with sodium hydroxide and water, dried and evaporated. The residue was purified by filtration through a short column of silica gel using chloroform as eluant. Pure (10) was obtained (1.85 - 1.95 g; 80 - 85%). M.p. 42-4°C. ^{1}H-nmr δ 7.95 (d, 1H, J = 6.0 Hz), 6.85 (d, 1H, J = 6.0 Hz), 3.95 (s, 3H), 3.85 (s, 3H). ^{13}C-nmr δ 159.7, 145.8, 143.1, 137.4, 108.0, 60.6, 56.3. Anal. Calcd for $C_7H_8BrNO_2$: C, 38.56; H, 3.71; N, 6.42. Found: C, 38.46; H, 3.61; N, 6.43.

3,3',4,4'-Tetramethoxy-2,2'-bipyridyl (Tetramethyl orelline), (11). A) To a stirred, deep blu solution of $NiCl_2$ $6H_2O$ (1.18 g) and triphenylphosphine (5.2 g) in DMF (25 ml) under nitrogen at 50°C, zinc powder (0.32 g) is added. After about 1 h, the colour of the mixture has changed to red brown. 2-Bromo-3,4-dimethoxy pyridine (10) (1.05 g) is added and the progress of the reaction is monitored by tlc. After 2 h the starting product has been consumed. The mixture is poured into dilute ammonia solution and extracted with chloroform; the organic layer is washed with water, dried and evaporated. The residue is chromatographed through a silica gel column using a 97 : 3 mixture of chloroform and methanol as eluant. Compound (11) was obtained in 75 - 87% (1.0 - 1.15 g). M.p. 187-9°C (Lit.[10] 186-7°C). ^{1}H-nmr δ 8.35 (d, 1H, J = 5.5 Hz), 6.9 (d, 1H, J = 5.5 Hz), 3.95 (s, 3H), 3.7 (s, 3H); (CD_3OD) δ 8.25 (d, 1H, J = 5.5 Hz), 7.2 (d, 1H, J = 5.5 Hz), 4.0 (s, 3H), 3.7 (s, 3H). ^{13}C-nmr δ 158.8, 150.9, 145.7, 144.1, 107.4, 61.0, 55.6; (DMSO-d_6) δ 158.2, 150.9, 145.1, 143.6, 108.2, 60.35, 55.8. Mass, m/e 277(M+1, 49), 261 (83), 259 (53), 245 (100), 231 (11), 201 (14). Anal. Calcd for $C_{14}H_{16}N_2O_4$: C, 60.85; H, 5.85; N, 10.14. Found: C, 60.65; H, 5.94; N, 9.98.

B) A mixture of (10) (0.5 g), electrolytic copper (0.4 g), potassium iodide (traces) in DMF (5 ml) was refluxed for 15 h. The cooled mixture is poured on water and extracted with chloroform. After work up, as described in A), the bipyridyl (11) was isolated in 8 - 10% yields.

C) A mixture of (10) (0.35 g), sodium formate (0.64 g), palladium on charcoal (0.05 g), cetyltrimethylammonium bromide (0.1 g), 32% sodium hydroxide (0.5 ml), and water (0.5 ml) is stirred and refluxed for 42 h. The cooled mixture is extracted with chloroform and worked up as in A). The desired bipyridyl (11) was obtained in 15 - 20% yields.

3,3',4,4'-Tetramethoxy-2,2'-bipyridyl-N,N'-dioxide (Tetramethyl orellanine), (19). A solution of (11) (0.6 g) and m-chloroperbenzoic acid (1.9 g) in chloroform (30 ml) is stirred at room temperature for 8 h. The solvent is evaporated and the solid residue is chromatographed through a silica gel column (deactivated with a 3% water) using a mixture of chloroform and methanol as eluant (in the ratio of 90 : 10 for the first fractions containing the acids and then in the ratio of 65 : 35). The dioxide (19) was obtained in 75 - 85% yields (0.5 - 0.57 g). M.p. 245-6°C dec. (Lit.[10] 235-6°C dec.). ^{1}H-nmr δ 8.05 (d, 1H, J = 7.0 Hz), 6.95 (d, 1H, J = 7.0 Hz), 4.0 (s, 3H), 3.9 (s, 3H). ^{13}C-nmr δ 153.4, 151.5, 146.5, 135.5, 109.3, 61.5, 56.4. Mass, m/e 309 (M+1, 12), 293 (M+1-O, 10), 277 (M+1-O_2, 100), 262 (58), 245 (45). Anal. Calcd for $C_{14}H_{16}N_2O_6$: C, 54.53; H, 5.24; N, 9.09. Found: C, 54.43; H, 5.31; N, 9.00.

3,3'-Dihydroxy-4,4'-dimethoxy-2,2'-bipyridyl (Dimethyl orelline), (20). A solution of the 3,3',4,4'-tetramethoxy-2,2'-bipyridyl (11) (0.24 g) and $BBr_3 \cdot SMe_2$ (2.15 g) in 1,2-dichloroethane (30 ml) was refluxed for 5 h. The cooled reaction mixture is poured on water and the pH is adjusted to 5 - 6 with dilute ammonia solution. The organic layer is separated and washed with water. The solvent is dried and evaporated and the residue is chromatographed through a silica gel column using chloroform as eluant. A yellow product (0.17 g; 80%), with a blue fluorescence under UV irradiation, was obtained. M.p. 252-4°C. ^{1}H-nmr δ 8.0 (d, 1H, J = 5.5 Hz), 6.85 (d, 1H, J = 5.5 Hz), 4.0 (s, 3H); (CD_3OD) δ 8.6 (d, 1H, J = 6.5 Hz), 7.8 (d, 1H, J = 6.5 Hz), 4.35 (s, 3H). ^{13}C-nmr δ 155.6, 146.4, 139.1, 136.9, 106.7, 56.0; (DMSO-d_6) δ 145.4, 137.3, 107.8, 55.8. Mass, m/e 249 (M+1, 100), 248 (M, 57), 234 (80), 232 (24), 231 (26), 220 (29), 203 (18), 178 (33), 163 (32), 151 (26), 137 (20), 109 (39), 60 (72), 58 (99). Anal. Calcd for $C_{12}H_{12}N_2O_4$: C, 58.05; H, 4.88; N, 11.29. Found: C, 58.21; H, 4.89; N, 11.16.

Compound (20) was recovered unchanged when it was treated with $BBr_3 \cdot SMe_2$, under conditions identical to those employed in the case of (8).

3,3',4,4'-Tetrahydroxy-2,2'-bipyridyl (Orelline), (3). A solution of the 3,3',4,4'-tetramethoxy-2,2'-bipyridyl (11) (0.4 g) in 48% hydrobromic acid was poured into a glass tube (5 ml). The protected sealed tube was left overnight into an oven at 150°C. The cooled vial contained large colourless crystals. The tube was carefully open and the excess hydrobromic acid was eliminated by distillation under vacuum. Water was added and then evaporated under vacuum. This operation was repeated for two or three times. The almost colourless solid residue was then dissolved in water and the pH was adjusted to 7 - 7.5 by slow addition of lithium carbonate. A yellow precipitate was formed which was collected by filtration. ^{1}H-nmr showed that the product obtained (0.16 - 0.19 g; 50 -60 %) was almost pure 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl (3). The

pure compound was obtained by sublimation at 0.01 mm/Hg and 220°C (BUchi GKR-50 sublimation apparatus). The bright yellow solid suffers decomposition when heated above 310 - 320°C. It is practically insoluble in all the most common organic solvents, but it dissolves in DMSO. ^{1}H-nmr (DMSO-d_6) δ 7.9 (d, 1H, J = 5.5 Hz), 6.9 (d, 1H, J = 5.5 Hz), 9.5 - 10.8 (broad s, 2H). ^{13}C-nmr (DMSO-d_6) δ 155.5, 145,8, 137.2, 136.4, 111.1. Mass, m/e 221 (M+1, 91), 203 (100), 192 (43.5), 151 (22), 138 (45), 137 (38), 136 (30), 110 (38), 95 (45), 70 (48). UV (MeOH) 216, 344, 390; (0.1 N HCl) 305, 248, 212. Anal. Calcd for $C_{10}H_8N_2O_4$: C, 54.54; H, 3.66; N, 12.72. Found: C, 54.35; H, 3.83; N, 12.58.

A small amount of compound (3) was stirred at room temperature with 2.5 molar equivalents of an ethereal solution of diazomethane. Examination by tlc showed that (3) was converted into a new yellow compound, which showed an intense blu fluorescence under UV irradiation. These properties, as well as the R_f, under several different conditions, were identical to those presented by compound (20). Addition of an excess of diazomethane produced the complete consumption of this compound and the appearance on the tlc plate of a new spot whose R_f, under several different conditions, was identical to that of the 3,3',4,4'-tetramethoxy-2,2'-bipyridyl (11).

As described below, orelline (3) was also obtained from orellanine (1) by sublimation.

3,3',4,4'-Tetrahydroxy-2,2'-bipyridyl-N,N'-dioxide (Orellanine), (1). A) Orelline (3) (0.2 g) was stirred at room temperature with excess 35% hydrogen peroxide (5 ml). The colour of the dispersed solid gradually changed from yellow to white. After 7 - 8 h most of the water was carefully evaporated under vacuum heating the temperature below 40°C. More water was added and removed by distillation. This operation was repeated for two or three times. A white crystalline product was obtained (0.18 - 0.2 g; 79 - 87%) which was identified as the 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl-N,N'-dioxide (orellanine) (1). This product is soluble in dilute sodium hydroxide solution, in ammonia and in DMSO; it is slightly soluble in methanol and insoluble in water and in all the most common organic solvents. This product is stable until 150 - 160°C; at higher temperatures it slowly decomposes to give the subliming yellow orelline (3). If the product is put on the plate of the melting point apparatus, kept at 270 - 280°C, it suffers an instantaneous and vigorous decomposition to give (3). ^{1}H-nmr (DMSO-d_6) δ 8.25 (d, 1H, J = 7.0 Hz), 7.15 (d, 1H, J = 7.0 Hz), 8.8 - 7.8 (broad s, 2H). ^{13}C-nmr (DMSO-d_6) δ 155.2, 150.6, 131.8, 130.1, 110.0. Mass, m/e 253 (M+1, 100), 237 (29), 221 (57), 204 (10), 192 (17), 164 (43), 138 (52), 137 (46), 110 (28), 108 (28), 95 (23), 71 (29), 55 (45). UV (MeOH) 216, 248, 279, 351; (0.1 N NaOH) 221 (sh), 232, 290, 315; (0.1 N HCl) 211, 262, 290. Anal. Calcd for $C_{10}H_8N_2O_6$: C, 47.62; H, 3.20; N, 11.11. Found: C, 47.91; H, 3.35; N, 10.86.

B) A mixture of 3,3',4,4'-tetramethoxy-2,2'-bipyridyl-N,N'-dioxide (19) (0.2 g) and 48% hydrobromic acid (3 ml) was stirred at 120°C for 5 h. The excess acid was removed by distillation under vacuum and the solid residue was taken up with water. Water was distilled off under vacuum; this operation was repeated for two or three times. The residue was dissolved with water and the solution was made alkaline by addition of dilute ammonia. The pH was adjusted to 5 by addition of acetic acid after which a white precipitate was formed. The solid was collected by filtration and dried under vacuum (0.09 - 0.1 g; 54 - 60%). This compound was identified as the 3,3'4,4'-tetrahydroxy-2,2'-bipyridyl-N,N'-dioxide (orellanine) (1); its physical and spectral data were identical to the sample obtained as described in A).

Sublimation of (1) under reduced pressure (0.01 mm/Hg, 220°C) produced the 3,3',4,4'-tetrahydroxy-2,2'-bipyridyl (orelline) (3) in 50% yields.

A white sample of (1) when exposed to sun light or to UV irradiation for few seconds gave the characteristic change of colour described for the natural orellanine.[5]

3,3'-Dihydroxy-2,2'-bipyridyl, (22). This compound was obtained in 60% yields by the demethylation of the 3,3'-dimethoxy-2,2'-bipyridyl[9] (21). The dealkylation was effected with $BBr_3 \cdot SMe_2$ under conditions identical to those described above for the synthesis of (20). M.p. 188-90°C (Lit.[19] 196°C). ^{1}H-nmr δ 8.05 (dd, 1H, J = 1.8 and 4.5 Hz), 7.4 (dd, 1H, J = 1.8 and 8.0 Hz), 7.25 (dd, 1H, J = 4.5 and 8.0 Hz). ^{13}C-nmr δ 156.3, 139.9, 136.0, 125.9, 124.9.

Acknowledgment. We gratefully acknowledge financial support from the CNR, Rome, and Ministero della Pubblica Istruzione, Italy.

REFERENCES and NOTES

1) This work is part of the Tesi di Laurea of Miss Cinzia Pierucci.
2) S. Grzymala, Rocz. PZH, 12, 491 (1961); Mikol Mitteilungsblatt, 3, 1 (1958); Bull. Soc. Myc. France, 78, 394 (1962).
3) W. Z. Antkowiak and W. P. Gessner, Bull. Acad. Polon. Sciences Sez. Chim., 23, 729 (1975).
4) W. Z. Antkowiak and W. P. Gessner, Tetrahedron Lett., 1931 (1979).
5) W. Z. Antkowiak and W. P. Gessner, Experientia, 41, 769 (1985).
6) The results of the researches carried out on *Cortinarius Orellanus* by other authors are summarized on references 2 and 4.
7) D. Wenkert and R. B. Woodward, J. Org. Chem., 48, 283 (1983).
8) W. Z. Antkowiak and W. P. Gessner, Tetrahedron Lett., 25, 4045 (1984).
9) M. Tiecco, L. Testaferri, M. Tingoli, D. Chianelli, and M. Montanucci, Synthesis, 736 (1984).
10) E. V. Dehmlow and H.-J. Schulz, Tetrahedron Lett., 26, 4903 (1985).
11) K. Lewicka and E. Plazek, Roczniki Chem., 40, 405 (1966); Chem. Abstr., 65, 7134g (1966).
12) P. Nedenskov, N. Clauson-Kaas, J. Lei, H. Heide, G. Olsen, and G. Jansen, Acta Chem. Scand., 23, 1971 (1969).
13) G. R. Newkome, D. C. Pantaleo, W. E. Puckett, P. L. Ziefle, and W. E. Deutsch, J. Inorg. Nucl. Chem., 43, 1529 (1981); G. R. Newkome, W. E. Puckett, G. E. Kiefer, V. K. Gupta, Y. Xia, M. Coreil, and M. A. Hackney, J. Org. Chem., 47, 4116 (1982); P. Bamfield and P. M. Quan, Synthesis, 537 (1978).
14) P. G. Williard and C. B. Fzyhle, Tetrahedron Lett., 21, 3731 (1980).
15) M. V. Bhatt and S. U. Kulkarni, Synthesis, 249 (1983).
16) E. C. Constable and K. R. Seddon, Tetrahedron, 39, 291 (1983).
17) J. G. Wirth, U. S. Patent, 3,676,448 (1972); 3,767,652 (1973); Chem. Abstr., 77, 103415 (1972); 80, 109920c (1974).
18) H. J. Den Hertog, F. R. Schepman, J. De Bruyn, and G. J. E. Thysse, Recueil, 69, 1281 (1950).
19) W. Siemanowski and H. Witzel, Liebigs Ann. Chem., 1731 (1984).