Enzymic Phenol Oxidation of *N*-Methylcoclaurine. Studies on the Syntheses of Heterocyclic Compounds. Part CCCXXXII (1)

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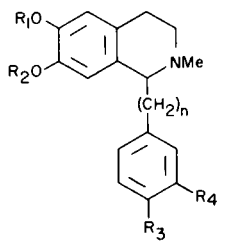
The enzymic phenol oxidation of *N*-methylcoclaurine (III) with homogenized potato peels in the presence of hydrogen peroxide was examined and found to give a mixture of the dimer (VI) and trimer (VII) with C–O–C head to tail coupling. Furthermore, the same oxidation of III with homogenized rhizome of *Nelumbo nucifera Gartner* and hydrogen peroxide was also investigated.

In a previous paper (2) we have reported that the enzymic phenol oxidation of 1,2,3,4-tetrahydro-7-hydroxyl-1-(4-hydroxyphenethyl)-6-methoxy-2-methylisoquinoline (I) with homogenized potato peels-hydrogen peroxide gave the promelanthioidine (II) as the dimer having a C–O–C head to tail coupling which has not yet been achieved *in vitro*. Therefore, the phenol oxidation of *N*-methylcoclaurine (III) under the same conditions as above was carried out, in order to examine whether the liensinine (IV) type or tubocurarine (V) type compounds would be formed or not, and thus we wish to report these interesting results.

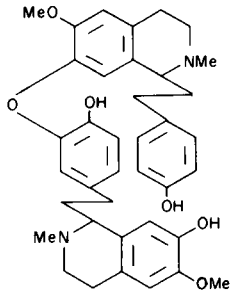
N-methylcoclaurine (3) (III) was oxidized with the potato peels-hydrogen peroxide system and after treatment of the reaction mixture as usual, a colorless powder (VI), m.p. 125-130° and another compound (VII), m.p. 160-165°, were isolated chromatographically. The mass spectral data of IV and VII support that the former is a C–O–C head to tail coupled dimer as shown in Scheme 2 and the latter is a C–O–C head to tail coupled trimer as depicted in Scheme 3. The other spectral and chemical data supported the above facts, which will be described in the experimental section.

On the other hand, phenol oxidation of III with homogenized rhizome of *Nelumbo nucifera Gartner*-hydrogen peroxide at *p*H 7.0 gave *p*-hydroxybenzaldehyde. This fact is similar to the formation of *m*-hydroxybenzaldehyde in the case of phenolic oxidation (4) of IIIa-c with horseradish peroxidase-hydrogen peroxide at *p*H 9.0. In order to compare the fragmentation patterns of the above dimer (VI) with those of neferine having the head to tail coupled structure (2), the modified extraction of neferine (5) (VIII) was carried out to give the free base which was acetylated to afford the acetyl derivative (IX). In the mass spectrum of IX, the following fragmentation patterns were shown in Scheme 4. Loss of one hydrogen radical from IX gave the ion at m/e 665 and the cleavage at the bond (a) afforded the ion at m/e 545, from which loss of CH_2CO group was observed as the ion at m/e 503. Furthermore, the ion at m/e 206, which was cleaved at the bond (c), was demethylated to give the ion at m/e 191. Loss of one hydrogen radical from the fragment ion, which was cleaved

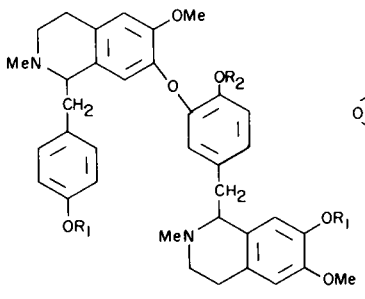
SCHEME 1

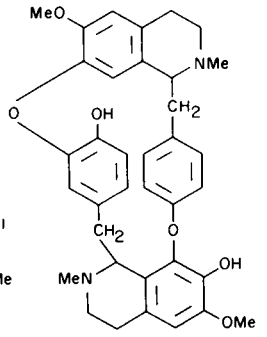


I: R_1 = Me, R_2 = R_4 = H, R_3 = OH, n = 2
III: R_1 = Me, R_2 = R_4 = H, R_3 = OH, n = 1
IIIa: R_1 = R_2 = R_3 = H, R_4 = OH, n = 1
IIIb: R_1 + R_2 = $-CH_2-$, R_3 = H, R_4 = OH, n = 1
IIIc: R_1 = R_2 = Me, R_3 = H, R_4 = OH, n = 1



(II)

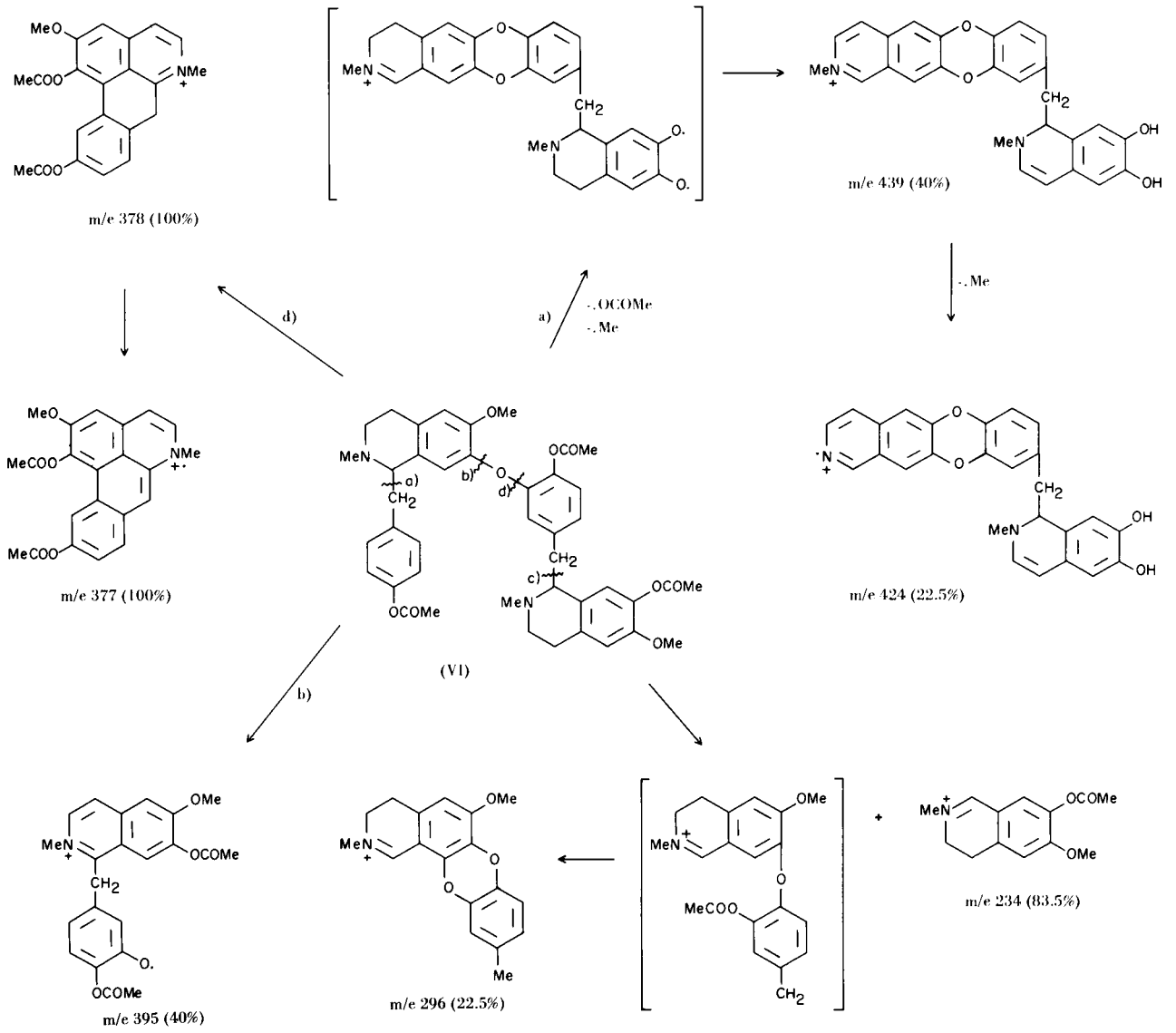




IV: R_1 = R_2 = H
VIII: R_1 = Me, R_2 = H
IX: R_1 = Me, R_2 = COMe

(V)

SCHEME 2

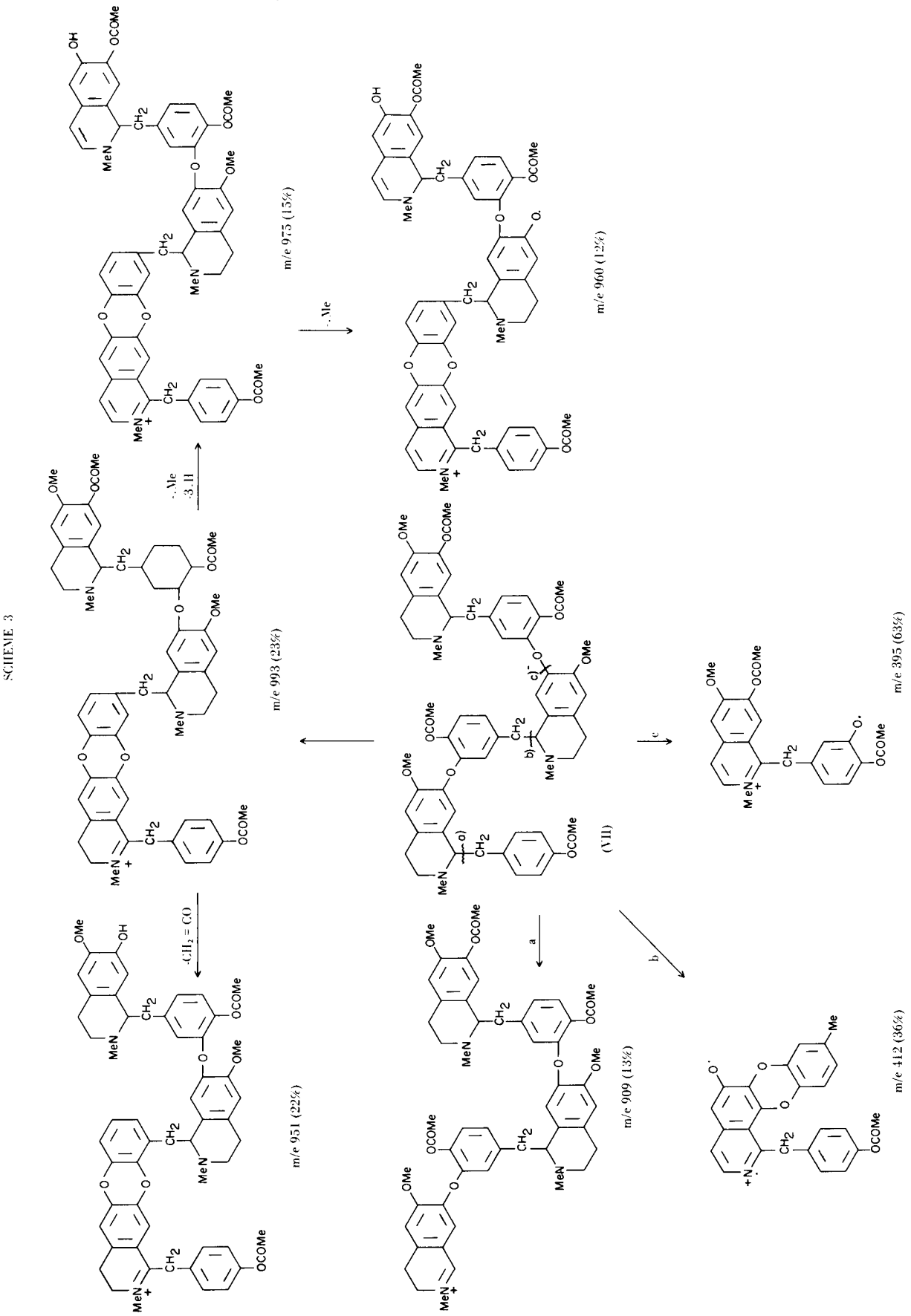


at the bond (d), gave the ion at m/e 353, from which expulsion of a methyl radical, followed by loss of CH_3CO group, afforded the ions at m/e 339 and 296, respectively. The cleavage of the bond (b) also gave the ion at m/e 369, from which a methyl radical was removed to give the ion at m/e 354. Further expulsion of an acetyl group gave the ion at m/e 312. Moreover, the cleavage between the bonds (a) and (c), followed by expulsion of the acetyl group, gave the ion at m/e 296. The compositions of each fragmentation ion were revealed by the high resolution mass spectrum as shown in Table I.

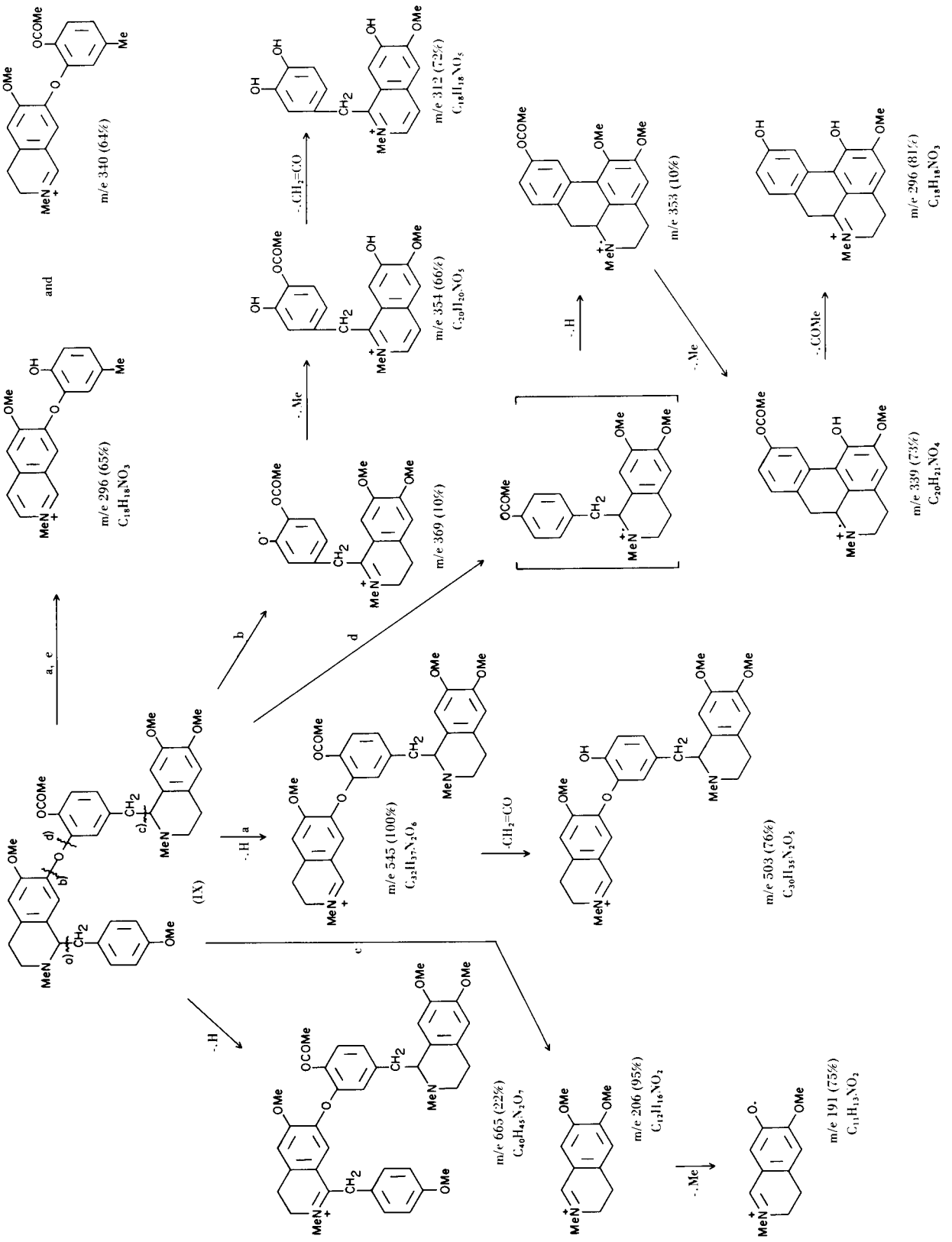
TABLE I

Mass number (*m/e*)	Formula	Observed	Calculated
655	$C_{40}H_{45}N_2O_7$	665.321	665.323
545	$C_{32}H_{37}N_2O_6$	545.264	545.265
503	$C_{30}H_{35}N_2O_5$	503.257	503.255
354	$C_{20}H_{20}NO_5$	354.134	354.134
339	$C_{20}H_{21}NO_4$	339.147	339.147
312	$C_{18}H_{18}NO_4$	312.125	312.124
296	$C_{18}H_{18}NO_3$	296.128	296.129
206	$C_{12}H_{16}NO_2$	206.118	206.118
191	$C_{11}H_{13}NO_2$	191.093	191.095

SCHEME 3


m/e 993 (23%)
m/e 975 (15%)
m/e 960 (12%)
m/e 951 (22%)
m/e 909 (13%)
m/e 395 (63%)
m/e 412 (36%)
(VII)
-Me
-3H
-CH2=CO
-Me
a
b
c

SCHEME 4


(IX)
a, e
b
d
-H
a
-CH2=CO
-Me
-H
-Me
-COMe
-H
-Me
and
m/e 340 (64%)
m/e 296 (65%) C18H18NO3
m/e 354 (66%) C20H20NO5
m/e 312 (72%) C18H18NO5
m/e 369 (10%)
m/e 545 (100%) C32H37N2O6
m/e 503 (76%) C30H35N2O5
m/e 665 (22%) C40H45N2O7
m/e 206 (95%) C12H16NO2
m/e 191 (75%) C11H13NO2
m/e 353 (10%)
m/e 339 (73%) C20H21NO4
m/e 296 (81%) C18H18NO3

Thus, it seems to be of great interest that the factor promoting the C–O–C head to tail coupling of 1-benzyl- (III) and 1-phenethylisoquinoline derivatives (2) would exist in the homogenized potato peels-hydrogen peroxide system. Further, it is also notable that no formation of dienones (6) have been observed in the enzymic oxidative coupling of *N*-methylcoclaurine as well as 1-phenethylisoquinoline (2).

EXPERIMENTAL (7)

Phenol Oxidation of *N*-Methylcoclaurine (III) with Homogenized Potato Peels in the Presence of Hydrogen Peroxide.

To a solution of 4 g. of *N*-methylcoclaurine (III) in a small amount of 10% acetic acid was added 2000 ml. of water and a suitable amount of ammonium acetate was added to the above solution to *p*H 4.8. A mixture of 2 ml. of 30% hydrogen peroxide and 100 g. of homogenized potato peels were then added to the above mixture, which was allowed to stand at room temperature (5-18°) for 8 days. During this time a mixture of 20 g. of homogenized potato peels and 1 ml. of 30% hydrogen peroxide was added every two days. After the reaction mixture had been basified with ammonia to *p*H 8.5 and filtered using celite, extraction of the filtrate with butanol, followed by evaporation, gave a brown solid, whose solution in 50 ml. of tetrahydrofuran was acetylated by shaking with 2 ml. of acetic anhydride in the presence of 2.5 g. of potassium carbonate at room temperature for 5 hours. After filtration, the filtrate was evaporated *in vacuo* to give the residue which was basified with aqueous sodium bicarbonate solution and extracted with chloroform. The extract was washed with water, dried over sodium sulfate and evaporated to give a brown syrup, which was chromatographed on silica gel (100 mesh) using chloroform-methanol (10:1).

Removal of the first eluate gave 18 mg. (0.39%) of the dimer (VI) whose thin layer chromatogram showed one spot, R_f 0.8 [Wakogel B-5, chloroform-methanol-hexane (2:0.6:1)]; infrared cm^{-1} (chloroform), 1740 (C=O), 2750 (NMe); nmr (deuteriochloroform) δ, 2.16 (6H), 2.14 (3H) (two singlets, 3 x $OCOCH_3$), 2.43 (6H, singlet, 2 x NCH_3), 3.69 (6H, singlet, 2 x OCH_3), 6.2-7.2 (11H, multiplet, aromatic protons); ultraviolet, λ max (ethanol) 274 mμ, mass spectrum (m/e): 439, 424, 234, 296, 395, 378, 377. Trituration of the above syrup with ether gave a colorless powder, m.p. 125-130°.

Anal. Calcd. for $C_{42}H_{46}O_9N_2 \cdot 3.5H_2O$ (8): C, 64.19; H, 6.79. Found: C, 64.11; H, 7.07.

Removal of the second eluate gave a pale brown syrup, which was triturated with ether to give 2.2 mg. (0.14%) of the trimer (VII) as a powder, m.p. 160-165°, mass spectrum (m/e): 993, 975, 960, 951, 909, 412.

Phenol Oxidation of III with Homogenized Rhizome of *Nelumbo nucifera Gartner* and Hydrogen Peroxide.

To a mixture of 1 g. of the hydrochloride of III, 100 ml. of water and 1 ml. of 30% hydrogen peroxide was added 100 g. of homogenized rhizome of *Nelumbo nucifera Gartner*, and, after shaking, the mixture was allowed to stand at room temperature (5-18°) for 4 days. After filtration with celite, followed by extraction with chloroform, the extract was dried over sodium sulfate and evaporated to give a brown syrup, which was chromatographed on silica gel (100 mesh) using chloroform-hexane (50:1) as an eluant. Removal of the solvent gave 7.8 mg. (21.%) of *p*-hydroxybenzaldehyde, m.p. 120°; infrared cm^{-1} (chloroform) 3470 (OH), 1650 (aldehyde C=O); nmr deuteriochloroform δ, 5.17 (1H, broad singlet, OH), 6.95, 7.18 (each 2H, a pair of doublets, J = 6.5 cps, aromatic protons), which was identical with an authentic sample.

Isolation of Neferine (VIII) from the Embryo of *Nelumbo nucifera Gartner.*

The embryo (242 g.) of *Nelumbo nucifera Gartner* was extracted with benzene-chloroform (1:1) in the presence of potassium carbonate and the extract was washed with water, dried over sodium sulfate and evaporated to give a residue, which was extracted with ether. Evaporation of the dried solvent gave a syrup, to whose solution in methanol was added perchloric acid. Collection of the precipitate, followed by recrystallization from methanol gave the liensinine perchlorate.

The above filtrate was basified with potassium carbonate to give a free base, which was treated as usual. The resultant free base in tetrahydrofuran was shaken with acetic anhydride at room temperature in the presence of potassium carbonate for 3 hours. After filtration, the filtrate was evaporated, basified with saturated sodium carbonate solution, and extracted with chloroform. The extract was washed with water, dried over sodium sulfate and evaporated to give a residue, which was chromatographed on silica gel (100 mesh).

Removal of the first chloroform-methanol (100:1) eluate gave 54 mg. of the acetyl derivative (IX), whose thin layer chromatogram showed one spot, 0.7 [Wakogel b-5, chloroform:methanol (5:1)]; infrared cm^{-1} (chloroform) 1750 (acetyl C=O); nmr (deuteriochloroform) δ, 2.17 (3H, singlet, $COCH_3$), 2.43 (6H, singlet, 2 x NCH_3), 3.56, 3.69, 3.70, 3.74 (12H, four singlets, 4 x OCH_3), 6.35-7.10 (11H, multiplet, aromatic protons); mass spectrum (m/e): 655, 545, 503, 354, 339, 312, 296, 234, 206, 191.

Secondly, a solution of 50 mg. of the preceding derivative (IX) in 10 ml. of 5% methanolic sodium hydroxide solution was refluxed on a water bath for 30 minutes and the solvent was then removed by distillation to afford a syrup, whose solution in 5 ml. of water was saturated with an excess of crystalline ammonium chloride. The resultant ammoniacal solution (*p*H 9.6) was extracted with chloroform. The extract was washed with water, dried over sodium sulfate and evaporated to give 38.4 mg. of the neferine as a colorless syrup; nmr (deuteriochloroform) δ, 2.47, 2.43 (each 3H, two singlets, 2 x NCH_3), 3.77, 3.71, 3.68, 3.52 (12H, four singlets, 4 x OCH_3), 6.98-6.11 (11H, multiplet, aromatic protons), 5.60 (1H, broad singlet, OH); $[\alpha]_D^{28}$ -42.6° (c = 1.5 in chloroform), whose methiodide, m.p. 179-182°, was identical with an authentic sample (7).

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(7) Infrared spectra were measured with a Hitachi EPI-3 recording spectrophotometer, nmr spectra with a Hitachi H-60 spectrometer with tetramethylsilane as internal standard, and high resolution mass spectra were taken on a Hitachi RMU-7 mass spectrometer. All melting points are uncorrected.

(8) This hygroscopic sample was dried on phosphorus pentoxide at 60° for 24 hours and the OH band due to water of crystallization was observed at 3400 cm^{-1}.

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