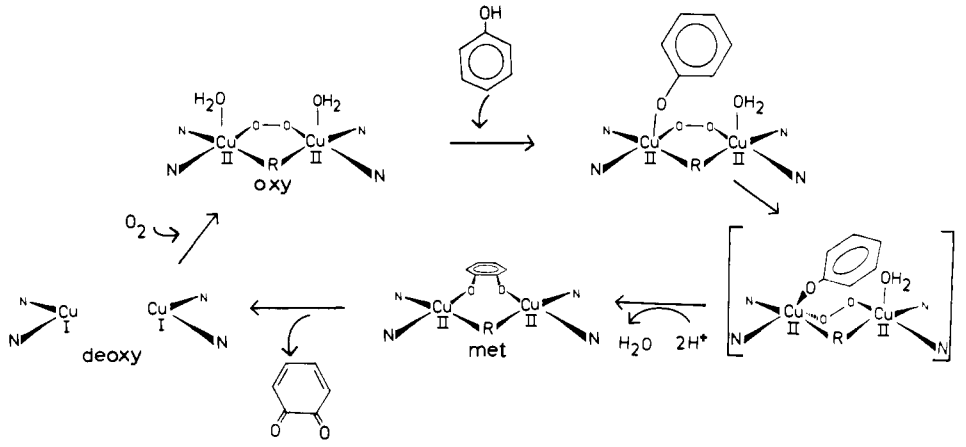
Scheme I. Proposed Mechanism of Hydroxylation and Oxidation of Phenols to Form *o*-Diquinones by *Neurospora* Tyrosinase



mimosine causes a new band to grow in at 350 nm ($\Delta\epsilon$ 2500 cm^{-1} M^{-1}), and "d–d" bands are observed centered around 700 nm.[11] No new bands are observed around 500 nm expected for a bridging N_3^-,[2] and the N_3^- is removed by passage through Sephadex G-25. Therefore N_3^- is weakly bound to the $^1/_2$-met site as would be predicted for group II behavior of mimosine.

Group II behavior requires that upon ligand binding the Cu(I)Cu(II) distance increases to > 5 Å.[5] This cannot be done by bridging the coppers through the two aromatic oxygens of mimosine and therefore suggests a geometry where one phenolate oxygen binds to the Cu(II) (required by the phenolate → Cu(II) CT transition at 430 nm) and perhaps the carboxylate binds to the Cu(I). The geometry around the Cu(II) must be quite distorted as evidenced by the rhombic splittings and perpendicular hyperfine structure in the EPR. A Cu(II)Cu(II) distance of >5 Å is not consistent with the lack of EPR signal in the met mimosine derivative. In this case the mimosine probably binds to the binuclear copper center through one or both aromatic oxygens.

After 24 h in the presence of 300-fold excess N_3^-, the $^1/_2$-met mimosine complex does convert to the $^1/_2$-met N_3^- form of tyrosinase as evidenced by EPR (Figure 2). As with the met mimosine derivative, N_3^- displaces mimosine, again indicating that N_3^-, mimosine, and peroxide all compete for the same binding site at the binuclear copper active site of tyrosinase.

In our earlier studies which probed small molecule binding to the tyrosinase site, a structural mechanism was proposed which predicted that both the small molecule and the organic substrate would first bind associatively to an axial position at the tetragonal cupric site and then rearrange through a trigonal-bipyramidal intermediate to the same equatorial binding site.[2] In this communication we have demonstrated that the competitive inhibitor mimosine does indeed bind equatorially to the binuclear copper center in both the met and the $^1/_2$-met derivatives and competes with the small molecules azide and peroxide for the same binding site. These results lead to the mechanism outlined in Scheme I as the pathway for hydroxylation and oxidation of phenols by tyrosinase. In an associative rearrangement through a trigonal-bipyramidal intermediate, the monophenol labilizes the peroxide from one copper, leaving a reactive polarized peroxide which can hydroxylate the phenol. Oxidation of the resulting diphenol would then occur from the equatorial position, and the *o*-quinone would likely leave dissociatively from the reduced binuclear cuprous site, allowing further turnover. Inhibitors, however, would not be oxidized and would therefore only be displaced from the active site by other exogenous ligands. It has been suggested that mimosine cannot displace O_2^{2-} from the binuclear copper center in hemocyanin because of poor accessibility of this site.[2] This suggestion would also be consistent with the above model of substrate binding to tyrosinase.

Acknowledgment. We are grateful to the National Institute of Arthritis, Metabolism and Digestive Diseases (Grant AM20406-05) and the Swiss N.S.F. (Grant 3.420.78) for support of this research. M.E.W. acknowledges the N.I.H. for a postdoctoral fellowship.

(11) From the equations $g_x = 2.0023 + 2\zeta/(E_{xy} - E_{xz})$ and $g_y = 2.0023 + 2\zeta/(E_{xy} - E_{yz})$ we estimate a 1500-cm^{-1} shift of the "d–d" bands to higher energy of $^1/_2$-met mimosine relative to the ternary complex of $^1/_2$-met mimosine and azide.

Novel Intramolecular Photorearrangement of Alkane Nitronate Anions

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Received March 31, 1981

We wish to report about the photorearrangement of alkane nitronate anions[1] which provides an interesting synthetic method of introducing hydroxamic acid functions. The nitronate anion **8**, produced from 2-nitro-1,7,7-trimethylbicyclo[2.2.1]heptane (**7**) in an EtOH–EtONa solution, showed a strong UV absorption at 240 nm (ϵ 12 800)[2] and was transformed to 2-hydroxy-1,8,8-trimethyl-2-azabicyclo[3.2.1]octan-3-one (**10**)[3] by irradiation with a low-pressure mercury lamp. Because it was reported that nitrones undergo photoisomerization to oxaziridines and then decompose to amides, this photoprocess was expected to involve *O*-oxaziridines (II)[4] (Scheme I).

A typical photoreaction procedure is as follows: A solution of 2-nitro-1,7,7-trimethylbicyclo[2.2.1]heptane (**7**) ($[\alpha]^{18}_D$ + 9.4° (EtOH))[5] (1.14 g) in EtOH–EtONa was irradiated for 4.5 h. After irradiation the solution was neutralized with 0.1 M AcOH in EtOH. Solvent removal followed by extraction from the residue with $CHCl_3$ gave a white solid (1.09 g) which was chromatographed[6] on silica gel to yield 0.71 g of *N*-hydroxy lactam **10**

(1) The formation of the nitronate anions largely depends on the kinetic acidity. Flanagan, P. W. K.; Amburn, H. W.; Stone, H. W.; Traynham, J. G.; Schechter, H. *J. Am. Chem. Soc.* **1969**, *91*, 2797.
(2) William, F. T., Jr.; Flanagan, P. W. K.; Taylor, W. J.; Schechter, H. *J. Org. Chem.* **1965**, *30*, 2674.
(3) An authentic sample prepared according to the literature was identical in all aspects with **10**. Nakazaki, M.; Naemura, K. *Bull. Chem. Soc. Jpn.* **1964**, *37*, 532.
(4) (a) Spence, G.; Taylor, C.; Buchardt, O. *Chem. Rev.* **1970**, *70*, 231. (b) Kikuchi, O.; Tanaka, S.; Naruchi, K.; Yamada, K. *J. Fac. Eng. Chiba Univ.* **1981**, *32*, 55.
(5) Forster, M. O. *J. Chem. Soc.* **1900**, *75*, 251.
(6) Flash chromatography was employed for the separation of all the photoproducts. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

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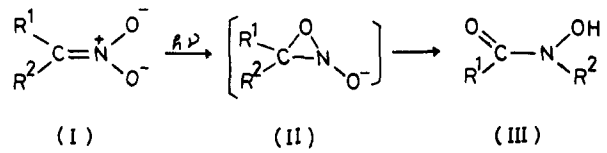
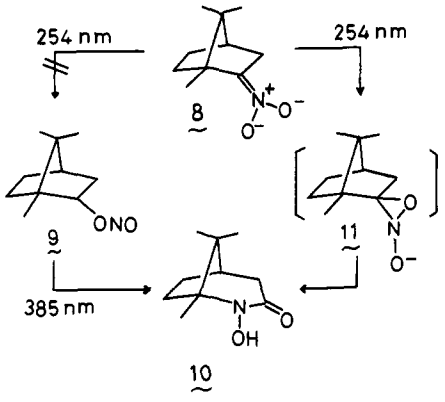
Scheme I



(I) (II) (III)

Scheme II

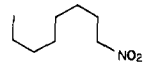
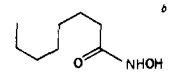
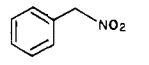
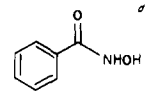
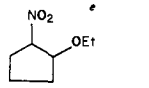
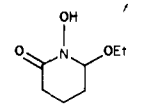
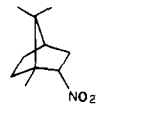
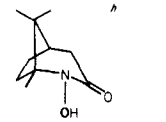
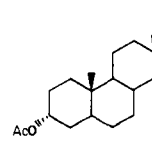
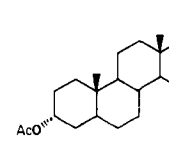


(76%), mp 212–214 °C, using $CHCl_3$–EtOH (20:1) as an eluant.

The results shown in Table I indicate that the highly substituted β carbon migrated to the nitrogen predominantly in the case of the anions of **5**, **7**, and **12**. A regioselective rearrangement was observed in the reaction of the anion of **5** and β carbon substitution by an ethoxy group dominated that on methylene to give 1-hydroxy-6-ethoxy-2-piperidone (**6**)[7] in 75% yield. The photoreaction of the anion of 3α-acetoxy-17β-nitro-5α-androstane (**12**)[8] ($[\alpha]^{25}_D$ +48.2° (dioxane)) proceeded stereoselectively to yield 17α-aza-*D*-homo-3,17a-dihydroxy-5α-androstan-17-one 3-acetate (**13**),[9] 78% ($[\alpha]^{20}_D$ −2.5° (dioxane)).[10] The byproducts were 3α-acetoxy-5α-17,18-cycloandrostane (**14**)[11] (17%), 3α-acetoxy-5α-androstane 17-oxime (**15**)[12] (4.0%), and 3α-acetoxy-5α-androstan-17-one (**16**)[12] (0.8%).

An alternative synthetic method to obtain the *N*-hydroxy lactam **10** is the Barton reaction in which the bornyl nitrite **9** was irradiated with monochromatic light (385 nm)[3] (Scheme II). Although bornyl nitrite shows a characteristic UV absorption at 360 nm, it was not identified spectroscopically in the course of the photoreaction of the nitronate anion **8**. This result ruled out the pathway proceeding via nitrite formation. Camphor **17** and camphoroxime **18** which were the byproducts[13] of the photoreaction of the anion **8** were expected to be produced from 2-nitro-1,7,7-trimethylbicyclo[2.2.1]heptane (**7**) by the reaction reported by Reid et al.[14]

Table I. Hydroxamic Acids from Nitronate Anions

nitroalkane	hydroxamic acid	yield,[a] %
 1	 2	
 3	 4	91[c]
 5	 6	75[g]
 7	 10	76[g]
 12	 13	78[k]

[a] Yields of purified products not optimized. [b] Reference 21. [c] $MeNH_2$–MeOH alkaline solution was employed.[19] [d] Reference 22. [e] Reference 23. [f] Reference 7. [g] EtONa–EtOH alkaline solution was employed. [h] Reference 3. [i] Reference 8. [j] Reference 9. [k] MeONa–MeOH alkaline solution was employed.

The well accepted fact that the hydroxamic acids are very soluble in water but less so in organic solvents[15] possibly minimized the yields of the hydroxamic acids **2**[16] and **4**[17] when EtOH–EtONa was selected as an alkaline solution. Methylamine which was reported to remove the protons from nitroalkanes effectively[18] was a useful base because the evaporation of the reaction mixtures took the amine away with the solvent.[19] Accordingly the yields of the hydroxamic acids **2** and **4**, which are almost threefold of those when EtONa was used as a base, were obtained as indicated in Table I.

The yields shown in Table I imply that this photoreaction proceeded quantitatively. This was also observed in the initial stage of the reaction of the nitronate anion **8**; in detail, the rate of disappearance of the anion **8** was followed spectroscopically and the rate of appearance of the *N*-hydroxy lactam **10** was followed by optical rotation. The data were treated as first-order kinetic and showed that the decrease in the quantum yield of the

(7) *N*-hydroxy lactam **6** was reduced by catalytic hydrogenation to give the lactam. The melting point was compared with the literature value for an authentic sample of 6-ethoxy-2-piperidone. Hubert, J. C. *Tetrahedron* **1975**, *31*, 1437.

(8) Nitroandrostane **12** was prepared by the method of Patchett et al.[24] The following spectral data were obtained for this nitrosteroid: mp 174–176 °C (from MeOH); IR 1745 ($\nu_{C=O}$), 1550 (NO_2 asym), 1380 (NO_2 sym), 1204 cm^{-1} (δ-C–O–C); NMR (CCl_4) δ 0.75 (18-H), 0.80 (19-H), 1.96 (3 H), 4.9 (1 H, 3β-H, $W_{1/2}$ = 6 Hz, eq), 4.24 (1 H, 17-H, $W_{1/2}$ = 17 Hz, ax). This new compound gave satisfactory elemental analysis.

(9) Mp, NMR, IR, MS, and $[\alpha]_D$ data for *N*-hydroxy lactam **13**, which turned to a reddish purple color with $FeCl_3$–HCl solution, were compared with the literature values for an authentic sample of 17α-aza-*D*-homo-3,17a-dihydroxy-5α-androstan-17-one 3-acetate. Robinson, C. H.; Gnoj, O.; Mitchell, A.; Wayne, R.; Kabasakalian, P.; Oliveto, E. P. *J. Am. Chem. Soc.* **1961**, *83*, 1771.

(10) Specific rotation of **13** was reported as $[\alpha]^{20}_D$ −2° (dioxane). Suginome, H.; Yonekura, N.; Mizuguchi, T.; Masamune, T. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 3010.

(11) (a) The following spectral data were obtained for cycloandrostane **14**: mp 98–103 °C (from EtOH–Et_2O), IR 1745 ($\nu_{C=O}$), 1240 (AcO), 1020, 1450 (δ-C–H); NMR (CCl_4) δ −0.04 (1 H), 0.29 (1 H), 0.56 (1 H), 0.80 (19-H), 2.06 (AcO), and 4.99 (1 H, $W_{1/2}$ = 7 Hz, eq, 3β-H).[11b] Typical cyclopropane ring proton signals appeared in the upper field.[11c] This new compound gave satisfactory elemental analysis. (b) Hydrolysis of **14** by NaOH–H_2O gave 3α-hydroxy-5α-17,18-cycloandrostane, which melted at 130–132 °C. The 3β-hydroxy isomer was reported to have a mp 153–155 °C.[11d] (c) Misiti, D.; Rosini, G.; Caglioti, L. *Gazz. Chem. Ital.* **1968**, *98*, 1284. (d) Half-height width of 3-H of the hydrolysis product was 10 Hz and indicated 3-H to be equatorial. Hassner, A.; Heathcock, C. *J. Org. Chem.* **1964**, *29*, 1350.

(12) Ruzicka, L.; Goldberg, M. W.; Meyer, J.; Brungger, H.; Eichenberger, E. *Helv. Chim. Acta* **1934**, *17*, 1395.

(13) Camphor **17** and camphoroxime **18** were identified by comparing the melting points with the literature values. Auwers, K. *Ber.* **1889**, *22*, 605.

(14) Reid, S. T.; Tucker, J. N.; Wilcox, E. J. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1359.

(15) Wise, M. M.; Brandt, W. W. *J. Am. Chem. Soc.* **1955**, *77*, 1058.

(16) The purified yield of **2** in the photoreaction in EtOH–EtONa was 30%.

(17) The purified yield of **4** in the photoreaction in EtOH–EtONa was 27%.

(18) Pearson, R. G. *J. Am. Chem. Soc.* **1948**, *70*, 204.

(19) A solution of $MeNH_2$–MeOH was prepared by bubbling the amine in cold MeOH and adjusting the pH at 12–13.

anion **8** was equal to the increase in quantum yield of the *N*-hydroxy lactam **10**.[20]

$$\Phi_{dec} = \Phi_{app}$$

Further investigation on the mechanism of this photoprocess including the electronic aspect of the excited anions is in progress.

(20) Quantum yields of the photoreaction of the anions **1**, **3**, **7**, and **12** were 0.1, 0.05, 0.02, and 0.01 in EtOH–EtONa, respectively.

(21) An authentic sample prepared according to the literature was identical in all aspects with **2**. Lewis, A. H. *Biochem. J.* **1927**, *20*, 1358.

(22) The hydroxamic acid **4** was identified by comparing with an authentic sample prepared by the method of Hauser et al. Hauser, C. R.; Renfrow, W. B., Jr. "Organic Syntheses"; Wiley: New York, 1943; Collect. Vol. II, p 67.

(23) 2-Ethoxynitrocyclopentane **5** was prepared as follows: 2-nitrocyclopentene (200 mg) was added to the solution of EtOH–EtONa (nitrocyclopentene:sodium = 1:1 in molar ratio) and stirred at room temperature. After 10 min of stirring, neutralization was carried out by 0.5 M HCl ethanolic solution followed by evaporation of ethanol and extraction by ether. Solvent removal from the extract gave a brown oil which was chromatographed on silica gel to yield **5** (65 mg), using benzene as an eluant. The following spectral data were obtained for this nitrocycloalkane **5**: NMR (CCl_4) δ 4.7 (1 H, sextet, J = 5 Hz), 4.3 (1 H, sextet, J = 5 Hz), 3.5 (2 H, q, J = 8 Hz), 2.3 (2 H, d, J = 8 Hz), 2.1–1.5 (4 H), and 1.2 (3 H, t, J = 8 Hz); IR (neat) 1550 (NO_2 asym) and 1370 cm^{-1} (NO_2 sym). This new compound gave satisfactory elemental analysis.

(24) Patchett, A. A.; Hoffman, F.; Giarrusso, F. F.; Schwam, H.; Arth, G. E. *J. Org. Chem.* **1962**, *27*, 3822.

A Micromethod for Determining the Branching Points in Oligosaccharides Based on Circular Dichroism

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Received July 20, 1981

As part of a new general approach for determining glycosidic linkages in oligosaccharides currently being developed in our laboratory, we describe a micromethod based on split CD curves of pyranose polybenzoates[1] which is suited for determining *glycosidic linkages at the branching points*. Despite the dramatic advancements made in structural analyses of polypeptides[2] and nucleic acids,[3] the complexity of oligosaccharide structures[4] has hampered the development of new microtechniques for structure determination. Although the sequencing of sugar units in oligosaccharides can be carried out by FD–MS,[5] the glycosidic linkage determination involves exhaustive methylation, hydrolysis and GC comparison of the monomeric methylated methyl glycosides with authentic specimens;[6] this identification process is severely restricted by the availability of standard samples. The method based on ^{13}C glycosidation shifts[7] is efficient and reliable but is applicable neither to samples of restricted supply nor to large molecules because of the appearance of ^{13}C NMR peaks in a narrow range.

A systematic investigation of more than 40 pyranose di-, tri-, and tetra-*p*-bromobenzoates clarified two aspects:[8]

(1) (a) Harada, N.; Nakanishi, K. *Acc. Chem. Res.* **1972**, *5*, 257. (b) Harada, N.; Nakanishi, K. "Circular Dichroic Spectroscopy—Exciton Coupling in Organic and Bioorganic Stereochemistry"; University Science Books: Mill Valley, CA, in press.

(2) (a) Morris, H. R. *Nature (London)* **1980**, *286*, 447. (b) Biemann, K. In "Biochemical Applications of Mass Spectrometry"; Walter, G. R., Dermer, O. C., Eds.; Wiley: New York, 1980; Suppl. Vol. 1, p 469.

(3) (a) Maxam, A. M.; Gilbert, W. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 560. (b) Sanger, F.; Nicklen, S.; Coulson, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5463.

(4) Three monomers XYZ give rise to 6 isomeric tripeptides; however, 1056 isomeric trisaccharides are conceivable: Clamp, J. *Biochem. Soc. Symp.* **1974**, *40*, 3. (As quoted by: Sharon, N.; Lis, H. *Chem. Eng. News* **1981**, *March 30*, 21.)

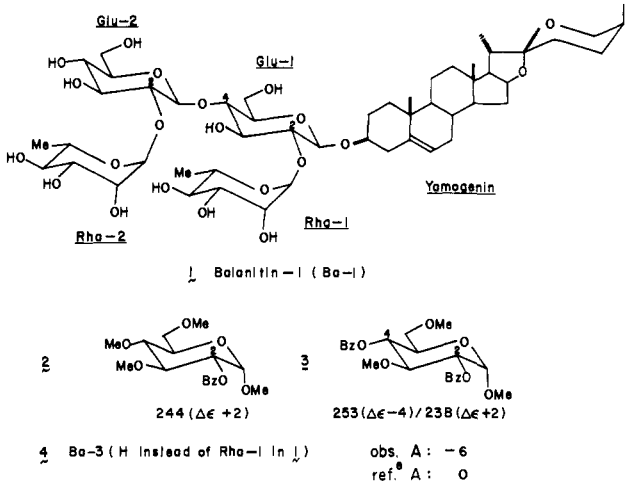
(5) Schulten, H. R.; Komori, T.; Kowasaki, T. *Tetrahedron* **1977**, *33*, 2595.

(6) Bouveng, H. O.; Lindberg, B. *Adv. Carbohydr. Chem.* **1960**, *15*, 53.

(7) (a) Kasai, R.; Suzuo, M.; Asakawa, J. I.; Tanaka, O. *Tetrahedron Lett.* **1977**, 175. (b) Tori, K.; Seo, S.; Yoshimura, Y.; Arita, H.; Tomita, Y. *Tetrahedron Lett.* **1977**, 179.

(8) Liu, H. W.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 5591.

Chart I



(i) The difference in $\Delta\epsilon$ values of the two extrema of split CD curves, or "*A* values", of *dibenzoates* may be regarded as constants which are solely dependent on spatial arrangements of benzoate groups on the pyranose ring, and to the extent that the conformation remains unaltered, the values are not affected by nonchromophoric substituents such as –OAc, –OMe, –O–Si-*t*-BuMe_2, –Me. Thus the following *A* values were obtained (the signs are determined by the chirality) for the reference di-*p*-bromobenzoates: 1,2-ee, 62; 1,2-ea, 62; 1,2-aa, 6; 1,3-ee, 0; 1,3-ea, 16; *A* values were also found for seven other dibenzoates involving the 6-benzoate.

(ii) The *A* values of a tribenzoate can be approximated by the sum of the three-component dibenzoate moiety. For example, the *A* value, +137, of methyl α-D-galactopyranoside-2e,3e,4a-tri-*p*-bromobenzoate, 253 nm ($\Delta\epsilon$, +95)/236 nm ($\Delta\epsilon$, −42), is equal to the sum of the three units, +62(2e,3e) + 62(3e,4a) + 16(2e,4a) = +140. The additivity approximation is still valid for tetrabenzoates consisting of six interacting dibenzoate units.

The present micromethod consists of submitting the oligosaccharide to permethylation, methanolysis, and benzoylation; the nonanomeric hydroxyl groups involved in glycosidic linkages are thus converted into *p*-bromobenzoyloxy groups. The *A* values of the di- and tribenzoates derived from the branched pyranose group(s) then establish the spatial arrangement of benzoate groups and hence those of the hydroxyl groups which were involved in the branching, *independent of the pyranose species and without reference to authentic samples*.

The method is exemplified by application to two new potent molluscicidal saponins, balanitin-1 (Ba-1) (**1**) and balanitin-2 (Ba-2) (**5**).[9]

The aqueous methanol extract of *Balanites aegyptiaca*, a popular East African medicinal tree,[10] exhibited insect antifeedant (Mexican bean beetle), antimicrobial (*B. subtilis*, *P. cruotosum*, and *S. cerevisae*), and molluscicidal activity. Fractionation of 1 g of the crude methanol extract by acetone extraction, LH-20 chromatography, and droplet counter-current chromatography,[11] as monitered by molluscicidal bioassay using *Biomphalaria glubratus*, a South American snail which is the host of schistosomes,[12] yielded 31 mg of Ba-1 (**1**), 10 mg of Ba-2 (**5**), and 15 mg of Ba-3 (**4**).

Ba-1 (**1**) (100 μg or 0.1 μmol) (Chart I) was permethylated with $CH_3I/Me_2SO/NaH$ (Hakomori method),[13] methanolyzed

(9) The structure of balanitin-1 could be determined by FD–MS and NMR spectroscopy; however, in the case of balanitin-2, the results from ^{13}C NMR data were not decisive: Liu, H. W.; Nakanishi, K. *Tetrahedron*, in press.

(10) (a) Watt, J. M.; Breyer-Brandwijk, M. G. "Medicinal and Poisonous Plants of Southern and Eastern Africa"; E.&S. Livingstone Ltd.: Edinburgh, **1962**; p 1064. (b) Kokwaro, J. O. "Medicinal Plants of East Africa"; East African Literature Bureau: Kampala, 1976; p 34.

(11) Hostettmann, K.; Hostettmann-Kaldas, M.; Nakanishi, K. *J. Chromatogr.* **1979**, *170*, 355 and references cited therein.

(12) Nakanishi, K.; Kubo, I. *Isr. J. Chem.* **1977**, *16*, 28.

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