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Oxidation of Benzoyl Methionine to Benzoyl Methionine Sulfoxide with 3-Hydroperoxyindolenines

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Benzoyl methionine (**4**), its methyl ester (**9**), and methionine have been oxidized with 3-hydroperoxyindolenines (**3a** and **7**) to the corresponding sulfoxides (**5**,**10**, and methionine sulfoxide).

Autoxidation of 3-alkylindoles (**1 a, b, c**) having a heteroatom at 2-position was found to undergo the direct hydroxylation at 3-position to give **6** (**a, b, c**)[2] and free radical chain mechanism involving a hydroperoxide as an intermediate was proposed.[3] In an extension of our work on autoxidation of indoles, we have found that 3-hydroperoxy-3-methyl-2-piperidinoindolenine (**3a**), which was generated by the *in situ* basification of HBr salt (**2**) in an open air at room temperature, oxidized isopropyl alcohol to acetone and Hantzsch ester (**12**) to 2,6-dimethyl-3,5-diethoxycarbonylpyridine (**13**), respectively.[3]

In further extension of our work we examined the oxidation of the methionine derivatives with 3-hydroperoxyindolenines. Numerous naturally occuring sulfoxides as well as methionine sulfoxide have been isolated.[4] However, the role of and the mechanism of the formation of these sulfoxides *in vivo* have not been known as yet.[5] A variety of oxidizing agent has been used for the oxidation of a thioether, but it is worthwhile to examine the oxidation of methionine derivatives with a reagent such as 3-hydroperoxyindolenine, which is considered most likely to exist in the biological system. It has been known that the 3-hydroperoxyindolenine is the possible intermediate of the biological oxidation of tryptophan to kynurenine.

An equimolar amounts of benzoyl methionine (**4**) and 3-methyl-2-piperidinoindolenine hydrobromide (**2**) in methanol was basified with sodium methoxide in an atmosphere of oxygen. The reaction proceeded at room temperature. Nearly one mole of oxygen was absorbed within 3.5 hr. The reaction mixture was treated with Dowex 50X8 to remove **6a** (82%). Preparative layer chromatography of the filtrate on silica gel with butanol-water-acetic acid gave benzoyl methionine sulfoxide (**5**) in 52% yield and 21% of **4** was recovered.

However, when two moles of **2** was used in the above reaction, two moles of oxygen-uptake took place within 3.5 hr. Apparently any appreciable amount of **4** could not be detected on thin-layer chromatography (TLC) and **5** was isolated in 63% yield after one crys-

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tallization. The benzoyl methionine sulfone which was usually produced by an excess of hydro gen peroxide, was not formed on this oxidation. Moreover, it is remarkable contrast that two moles of sodium cumyl hydroperoxide oxidizes dimethyl sulfide to the sulfone.[6]

Authentic benzoyl DL-methionine-*dl*-sulfoxide was prepared by the standard Schotten-Bauman reaction of DL-methionine-*dl*-sulfoxide.[7] The two diastereomers of benzoyl DL-methionine-*dl*-sulfoxide could easily be separated by fractional crystallization from water or ethanol. The less soluble crystals **5a** of one form which, on recrystallizations from water, melted at 181—182°.[8] Infrared (IR) and nuclear magnetic resonance (NMR) spectral data of this isomer are following. ν_{max}^{KBr} cm^{-1}: 3380 (NH), 1720 (COOH), 1655 (CONH), 990 (SO). NMR δ (100 MHz, in pyridine-d_5): 2.48 (3H, s, Me-S), 2.70 (2H, m, C-CH_2-C), 3.05 (2H, m, S-CH_2), 5.27 (1H, m, CH). From the mother liquor the other diastereomer **5b** was isolated, which after recrystallization from water, melted at 178—180°. ν_{max}^{KBr} cm^{-1}: 3220 (NH), 1735 (COOH), 1655 (CONH), 990 (SO). NMR δ (pyridine-d_5): 2.50 (3H, s, Me-S), 2.72 (2H, m), 3.12 (2H, t), 5.33 (1H, m). The mixed melting point of two racemates **5a** and **5b** gave 161—162°. Benzoyl methionine sulfoxide (**5**) obtained by the oxidation with **3a** was estimated to consist of a roughly 1:1 mixture of diastereomers by the fractional recrystallizations. The IR spectrum of the crude sulfoxide (**5**) was very similar to that of a 1:1 mixture of authentic diastereomers (**5a** and **5b**). The treatment of **4** in the reaction condition without **3a** did not give **5** and **4** was recovered unchanged.

Better result was obtained when 3-hydroperoxy-3-methyl-2-phenylindolenine[9] (**7**) was used instead of **3a**. A mixture of equimolar amounts of **4** and **7** in methanol was stirred for 5 hr at room temperature. Upon chromatographic separation, **5** was obtained in 60% yield after one crystallization and **8** was isolated in 85% yield. In absence of **7**, benzoyl methionine was not oxidized in the reaction condition. The oxidation of the methyl ester (**9**) with one mole of **7** proceeded more rapidly (*ca.* 3 hr) to give the sulfoxide (**10**) quantitatively. The 1:1 ratio of two diastereomers was determined by the comparison of two Me-S signals in the NMR spectrum. The use of two moles of **7** did not give the sulfone and **10** was obtained as a sole product. Small amounts of two by-products were isolated in the reaction of **9** with **7**. The formation of **11** from **7** has been reported.[9] The second minor product, 2-phenylskatole may be obtained by elimination of oxygen from **7**.[10] In analogous reaction condition, methionine itself has been oxidized to methionine sulfoxide, which was confirmed by paper chromatography as well as TLC.

Furthermore, the reaction of **4** and **7** in 50% triethylamine in methanol was carried out. Only 30% of **5** was obtained with 44% recovery of **4** and 86% of **8** has been isolated. The low yield of **5** provide an information that the sulfoxide formation competes with the oxidation of triethyl amine to triethylamine oxide.[11]

The marked differences in the reactivity between **3a** and **7** was obtained in the reaction of the Hantzsch ester (**12**). In contrast to the reaction of **12** with **3a**,[3] the oxidation of **12** by **7** did not take place and 67% of **7** was recovered. In addition, **7** was not able to oxidize the thioether moiety of **1c**, whereas **3c** and **3a** oxidized **1c** to give 2-ethylsulfinyl-3-methylindole as reported previously.[3] The hydrogen peroxide oxidation of **1c**,[12] however, gave the sulfone in 6% yield beside the sulfoxide as the main product (77%) at room temperature.

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11) **7** has been decomposed to **8** by triethyl amine[2] and triethyl amine oxide has been isolated (unpublished data).
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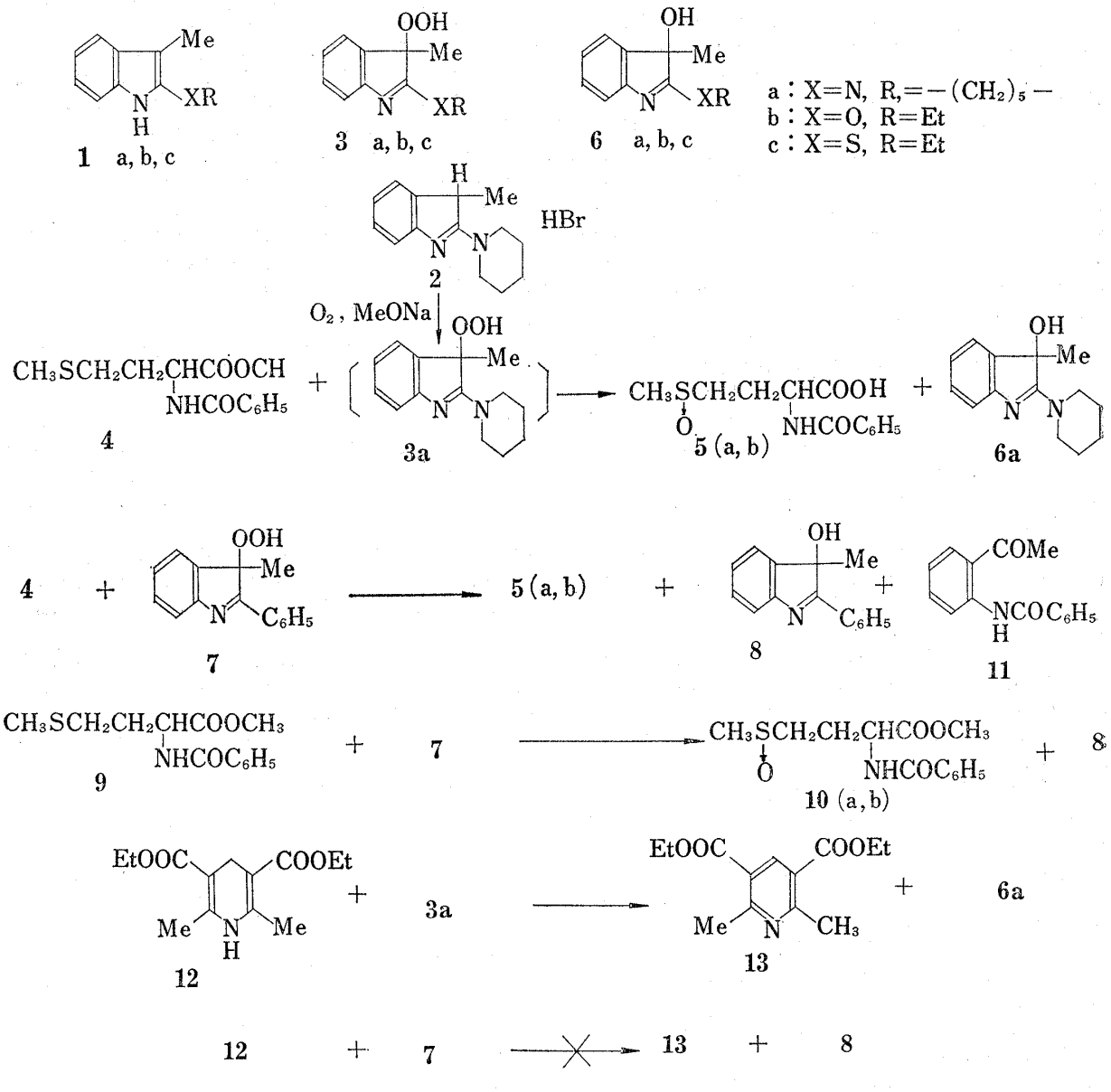
These results suggest that the substituent at 2-position effects the reactivity of 3-hydroperoxyindolenines, which oxidizes more selectively than hydrogen peroxide.



Experimental[13]

N-Benzoylmethionine Sulfoxide (5a and 5b)——i) From Methionine Sulfoxide: To a solution of methionine sulfoxide (15 g, 0.09 mole) and $NaHCO_3$ (7.8 g, 0.93 mole) in H_2O (210 ml) was added with cooling and stirring benzoyl chloride (37.9 g, 0.28 mole) in a small portions. The reaction mixture was kept at room temperature and unreacted benzoyl chloride was extracted with benzene. The aqueous layer was acidified with conc. HCl. The precipitates were collected and washed with benzene to remove benzoic acid, crude yield of **5**, 13.5 g (55.4%), which was recrystallized from a small amount of H_2O to give the first crop, 3.8 g of **5a**, mp, 179—181°. Recrystallizations from H_2O gave mp 181—182° (lit.[8] 170—171°). UV λ_{max}^{EtOH} nm

13) Melting points are uncorrected. IR spectra were recorded on HitachiG-3 model and Hitachi 215-spectrometers. UV spectra were recorded in 95% EtOH on Hitachi EPS-3T spectrophotometer. NMR spectra were determined in $CDCl_3$ or pyridine-d_5 with JOEL JNM4H-100 spectrometer with TMS as internal standard. The chemical shift was expressed by the δ-value in ppm. Mass spectra were obtained on a Hitachi RMU-6E mass spectrometer.

(ε); 228 (12200). Mass Spectrum: *m/e* (rel. intensity); 269 (0.78, M^+), 254 (1.44), 206 (9.4), 179 (8.6), 161 (12.5), 160 (8.2), 105 (100). IR and NMR: See text. *Anal.* Calcd. for $C_{12}H_{15}O_4NS$: C, 53.51; H, 5.61; N, 5.21. Found: C, 53.44; H, 5.67; N, 5.38.

Concentration of the mother liquor gave the second crop (2.8 g) as a mixture of two diastereomers. The third crop, the more soluble diastereomer, 4.0 g, mp 177—180°, was obtained by further concentration of the mother liquor. Recrystallizations from H_2O gave **5b**, mp 178—180°. UV: λ_{max}^{EtOH} nm (ε); 228 (12000). Mass Spectrum: *m/e* (relative intensity); 269 (1.1, M^+), 254 (1.3), 206 (7.2), 179 (4.3), 161 (9.0), 160 (9.0), 105 (100). IR and NMR spectral data: Ssee text. *Anal.* Calcd. for $C_{12}H_{15}NO_4S$: C, 53.51; H, 5.61; N, 5.21. Found: C, 53.22; H, 5.76; N, 5.40. The mixed melting point of two racemates **5a** and **5b** gave mp 161—162°.

ii) Oxidation of **4** by **3a** (1 mole): A solution of N-benzoylmethionine (**4**) (254 mg, 1 mM) and **2** (295 mg, 1 mM) in MeOH (20 ml) was placed in a closed vessel (hydrogenation apparatus) and oxygen was introduced and MeONa (162 mg, 3 mM) in MeOH (3 ml) was added with stirring by use of injector. Nearly 1 mole of O_2 was absorbed after 3.5 hr. The reaction mixture was filtered through Dowex 50×8. The elution with 20% MeOH was concentrated to give an oil (300 mg) which was separated by preparative layer chromatography on silica gel with BuOH–H_2O–AcOH into four fraction, A,B,C and D. The most polar fraction A was crystallized from H_2O containing a few drops of conc. HCl[14] to give **5** (120 mg, 45%), mp 163—165°, whose IR spectrum was very similar to that of a 1:1 mixture of two diastereomers. Recrystallization of next fraction B from H_2O–HCl gave **4** (53 mg, 21%), mp 146—149°. The fraction C (20 mg, 7%) was corresponded to that of N-benzoylmethionine sulfoxide methyl ester (**10**) which has been formed during Dowex column treatment with MeOH; Total yield of **5**, 52%. The fraction D (10 mg, 3.4%), mp 84—87°, was identified as **9** by comparison of the IR spectrum with that of the authentic material and the mixed mp 84—86°. The Dowex column was then eluted with NaOH–H_2O–MeOH. The eluate was concentrated and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed, dried and evaporated to give **6a** (189 mg, 82%), mp 205—206°[2] (from acetone).

iii) Oxidation of **4** with **3a** (2 moles): A solution of **4** (253 mg, 1 mM) and **2** (590 mg, 2 mM) in MeOH (20 ml) was added MeONa (216 mg, 4 mM) in MeOH (4 ml) and the reaction mixture was stirred for 3.5 hr. until O_2-uptake ceased. Workup as described above gave **5** (167 mg, 62%) and **4** (30 mg, 12%) was recovered. No sulfone was isolated.

iv) Control Experiment: A solution of **4** (130 mg, 0.5 mM) in MeOH (20 ml) was placed in a closed vessel and MeONa (81 mg) in MeOH (1.5 ml) was injected. The stirring was continued for 3.5 hr without any O_2-uptake and the starting material **4** was recovered in 96% yield.

v) Oxidation of **4** by **7**: A solution of **4** (127 mg, 0.5 mM) and **7** (125 mg, 0.5 mM) in MeOH (20 ml) was stirred at room temperature in an open air. The spot corresponded to 4 on TLC was completely disappeared after 5.5 hr and was converted to that of **5**. The evaporation of MeOH gave an oil (290 mg) which was separated by preparative layer chromatography on silica gel with CH_2Cl_2–MeOH (4:1). The more polar fraction was extracted with MeOH and crystallized from H_2O–conc. HCl to give **5** (81 mg, 60%), whose IR spectrum indicated a mixture of two diastereomers. The less polar fraction was extracted with MeOH to give **8** (100 mg, 86%), which was identical in comparison (IR, mixed mp) with authentic specimen prepared by $NaBH_4$ reduction of **7**.[9]

vi) Oxidation of **4** by **7** in the Presence of Triethyl Amine: A solution of **4** (253 mg, 1 mM) and **7** (249 mg, 1 mM) was dissolved in 20 ml of Et_3N–MeOH (1:1) and stirred for 5.5 hr at room temperature. Removal of the solvent left an oil (580 mg) which was separated by TLC on silica gel with CH_2Cl_2–MeOH (4:1) into three fractions. The most polar fraction was extracted with MeOH and crystallized from H_2O–conc. HCl to give **5** (80 mg, 30%). The next fraction gave **4** (100 mg, 44%) after crystallization from H_2O–HCl. The least polar fraction gave **8** (199 mg, 85%), mp 145—145.5° (from hexane–benzene).

N-Benzoyl Methionine Methyl Ester (9)——A solution of **4** (5.0 g) in MeOH (100 ml) saturated with dry HCl was refluxed for 1.5 hr to give **9** (4.4 g, 80%), mp 87.5—88° (recrystallized from MeOH). IR ν_{max}^{KBr} cm^{-1}: 3260 (NH), 1745 (COOMe), 1635 (CONH), 1225, 1175 (C-O-C). NMR (in pyridine-d_5); 1.95 (s, SMe), 2.25 (m, C-CH_2-C), 2.70 (t, S-CH_2), 3.62 (s, CO_2CH_3). Mass Spectrum: *m/e* 267 (M^+). *Anal.* Calcd. for $C_{13}H_{17}O_3NS$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.23; H, 6.43; N, 5.16.

N-Benzoylmethionine Sulfoxide Methyl Ester (10a,b)——i) Oxidation of **9** by **7** (1 mole): To a solution of **9** (506 mg, 1.9 mM) in MeOH (20 ml) was added **7** (478 mg, 2.0 mM) in MeOH (40 ml) and the reaction mixture was stirred for 3 hr under N_2. The solvent was evaporated and the residue (1.0 g) was column chromatographed on silica gel (86 g). The first elution with CH_2Cl_2 gave **10** (540 mg, 100%) as an oil, which was shown to consists of two diastereomers by NMR spectrum. IR ν_{max}^{film} cm^{-1} 1730 ($COOCH_3$), 1640 (NHCO), 1040 (SO). NMR (in $CDCl_3$): 2.3—2.7 (m, C-CH_2-C), 2.7—3.0 (m, S-CH_2), 2.57 (s, Me-SO), 2.58 (s, Me-SO), 3.8 (s, CO_2Me), 4.88 (m, CH), 7.4—8.0 (m, aromatic H). Mass Spectrum: *m/e* 283 (M^+).

A solution of crude **10** (430 mg) and MeOH–aq.NaOH (50 ml) was refluxed for 70 min. Workup in the usual way gave **5** (228 mg, 56%), mp 165—171°. Recrystallization from H_2O gave less soluble isomer

14) Extraction of **5** from Silica gel GF_{254} or Silica gel G with MeOH resulted in formation of some metal complex with **5**.

5a, mp 176—182°, identified (IR) with an authentic sample.

ii) Control Experiment: A solution of **9** (146 mg), in MeOH (15 ml) was stirred for 3.5 hr under N_2 and then stirred for another 13.5 hr in an open air. The TLC examination gave an only one spot corresponded to the starting material. Upon evaporation of the solvent **9** (148 mg, 100%), mp 85—86.5° was recovered and identified by IR and TLC.

iii) Oxidation of **9** by **7** (2 moles): To a solution of **9** (253 mg, 0.9 mM) in MeOH (20 ml) was added (478 mg, 2 mM) in MeOH (40 ml) and the reaction mixture was stirred for 3 hr under N_2. The residue (743 mg) obtained upon evaporation of the solvent was treated with CH_2Cl_2 and insoluble solid (134 mg), mp 150—155°, was separated and identified as **7**. The CH_2Cl_2 extract was subjected to preparative TLC on silica gel with hexane–ethyl acetate (4:1) into three fractions, A,B, and C. The most polar fraction (A, 250 mg, 92%) was identified as **10** by IR and TLC. The crude **10** was hydrolyzed with NaOH–aq.MeOH (30 ml) for 1 hr. The alkaline solution was filtered through Dowex 50×8 and eluted with 80% MeOH. The filtrate was evaporated to give **5** (194 mg). Recrystallization from H_2O gave less soluble isomer (**5a**, 44 mg), mp 17—175°, as the first crop and a mixture of **5a** and **5b** as the second crop (112 mg), mp 154—161°.

The next fraction (B) gave **7** (66 mg, total 200 mg, 42%). The third fraction (C, 196 mg, 42%,), mp 137—144°, was recrystallized from benzene–hexane to give mp 146—148°, which was identified as **8**. The least polar fraction gave *o*-benzoylaminoacetophenone (**11**, 15 mg).

An Attemped Oxidation of 12 by 7——i) To a solution of **7** (120 mg, 0.5 mM) in MeOH (20 ml) was added a solution of Hantzsch ester (**12**, 125 mg, 0.5 mM) in MeOH (20 ml). This solution was stirred for 45 hr at room temperature in an open air and the solvent was removed *in vacuo* to give a residue (234 mg), to which 2 ml of benzene was added. The insoluble solid was collected and identified as **7** (78 mg, 67%), mp 149—152° (ethyl acetate). The benzene soluble portion (152 mg) was subjected to preparative layer chromatography on silica gel developed with benzene–ethyl acetate (9:1) and **8** (9 mg, 7.8%), mp 145—147° (hexane–benzene) was obtained from the most polar fraction. The next fraction gave **11** (21 mg, 18%), mp 92—95° (benzene–hexane). The least polar fraction yielded **13** (103 mg, 79%), mp 68—69°, and 2-phenylskatole (8 mg, 6.9%).

ii) Control Experiment: A solution of **7** (121 mg) was dissolved in MeOH (20 ml) and stirred for 46 hr at room temperature in an open air and the solvent was evaporated to dryness to leave a residue, to which benzene was added. The insoluble solid (66 mg), mp 145—154°, was identified as **7**. The benzene extract was subjected to preparative TLC on silica gel developed with benzene–ethyl acetate (9:1) and **7** (14 mg) was isolated (total yield of **7**; 80 mg, 75%). The less polar fractions afforded **8** (10 mg, 9.3%) mp 138—140°, and **11** (9 mg, 8.4%), mp 92—95°, and 2-phenylskatole (8 mg, 7.5%).

iii) Control Experiment: A solution of **12** (131 mg) in MeOH (20 ml) was stirred for 46 hr at room temperature in an open air. To the residue obtained upon evaporation of the solvent was added MeOH and **12** (70 mg), mp 178—187° (MeOH) was obtained as the insoluble solid. The MeOH extract (42 mg) was subjected to preparative TLC on silica gel developed with hexane–acetone (4:1). The more polar fraction gave **12** (19 mg, total 89 mg, 75%). The less polar fraction gave **13** (30 mg, 25%), mp 69—72° (MeOH–H_2O).

The Reaction of 12 with 7 in the Presence of Triethylamine——A solution of **12** (127 mg, 0.5 mM), **7** (120 mg, 0.5 mM), and triethylamine (50 mg, 0.5 mM) in MeOH (40 ml) was stirred for 46 hr under N_2. The residue (282 mg) obtained upon evaporation of the solvent was separated by TLC on silica gel developed with hexane–ethyl acetate (4:1). From the most polar fraction, triethyl amine N-oxide (52 mg, colorless oil) was obtained and identified as its picrate, mp 163.5—166° (lit.,[15] mp 165° dec.), which was identical in comparison (IR, NMR, mixed melting point) with an authentic specimen. The next polar fraction gave **12** (98 mg, 77%) and then **7** (9 mg, 7.5%), **8** (68 mg, 61%), **13** (8 mg, 6.4%) were obtained. The less polar fraction gave **11** (6 mg, 5%) and 2-phenylskatole (2 mg, 2%).

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