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Oxidative Coupling of DOPA with Resorcinol and Phloroglucinol: Isolation of Adducts with an Unusual Tetrahydromethanobenzofuro[2,3-d]azocine Skeleton

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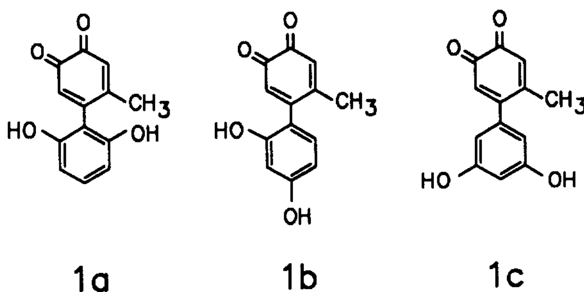
Abstract: *Under biomimetic conditions, both phloroglucinol and resorcinol cause significant inhibition of the oxidative conversion of dopa to dopachrome, a key step in the biosynthesis of melanin pigments. The major product (λ_{max}= 377 nm) from oxidation of dopa in the presence of phloroglucinol was characterized as 2, containing the unusual tetrahydromethanobenzofuroazocine ring system. Another product, formed in somewhat smaller amount, was formulated as 3, arising from coupling of phloroglucinol with 2 dopaquinone derived units. Analogous cross coupling adducts 4 and 5 were obtained by reaction of dopa or dopa methylester with resorcinol, respectively. These results suggest the trapping of labile dopaquinone, generated in situ from oxidation of dopa, by the reactive phenolic compounds, leading to the novel adducts 2, 4 and 5.*

A great number of *o*-quinones play critical roles in a variety of oxidative transformations occurring in living organisms such as the metabolism of catecholamines, particularly adrenaline, sclerotization of insect cuticles, and the defensive responses elicited by oxidative stress injuries.[1,2] However, the more simple quinones appear only transiently in the course of the oxidation process, and proof of their actual existence is usually prevented by the high instability exhibited. Among these, dopaquinone is of particular interest in relation to its key role in the oxidative conversion of tyrosine into melanins, the primary pigments of skin and hair.[2,3] This *o*-quinone is characterized by a marked tendency to undergo intramolecular cyclization.[3] Recently, Omote et al. reported the

trapping of dopaquinone generated from cerium(IV) or *o*-chloranile oxidation of dopa by treatment with cyclopentadiene,[4] phenylenediamine[5] or 1,2,3,4-tetrahydrocyclopent[b]indole.[6] It should be noted, however, that these reactions were performed at low temperatures, in organic solvents or two phases systems, and, in some cases, derivatization of dopa or the product resulting from trapping was required.

We now report on the oxidation of dopa under biomimetic conditions and on the direct isolation of cross coupling products arising from the trapping of dopaquinone by activated diphenols such as resorcinol and phloroglucinol. Our study was prompted by an earlier observation of the in vitro inhibition of some reactions involved in insect cuticle sclerotization.[7] In that case, coupling of the natural sclerotization agent N-acetyldopamine with its analog 2-hydroxy-N-acetyltyramine yields mixtures of biphenyls and dibenzofurans.[8] Similarly, Wong et al.[9] had reported on the reactions of 4-methyl catechol with a series of resorcinol derivatives promoted by clingstone peach polyphenol oxidase. The resulting products were tentatively formulated as adducts of type 1a-c, as direct characterization was hampered by their high instability.



In preliminary experiments, the course of melanogenesis in vitro, i.e. the tyrosinase catalyzed oxidation of dopa in aqueous buffer at neutral pH in the presence of either resorcinol or phloroglucinol was followed spectrophotometrically by monitoring the rate of formation of dopachrome chromophore at 475 nm.[10] As shown in fig. 1, both phloroglucinol (curve B) and resorcinol (curve C), at a molar ratio 5:1, prevent the formation of dopachrome significantly as compared with the control reaction (curve A).

HPLC and paper chromatographic analysis of the incubation mixture in the presence of either of the *m*-diphenolic compound, 30 min after addition of the enzyme, revealed the presence of a major component with λ_{max}=380 nm and a characteristic green fluorescence, along with minor amounts of 5,6-dihydroxyindole, arising from dopachrome rearrangement.[3] Appropriate

control experiments provided evidence that formation of the 380 nm chromophore did not result from tyrosinase catalyzed homocoupling of the phenolic compound. Moreover, no formation of the yellow compound was detected after mixing of dopachrome, prepared by ferricyanide oxidation of dopa, with either resorcinol or pholoroglucinol.

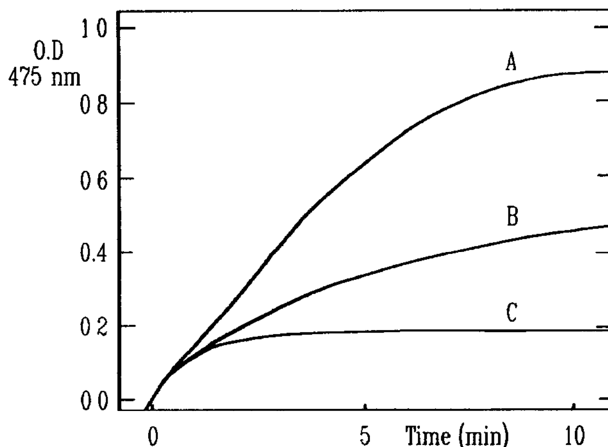


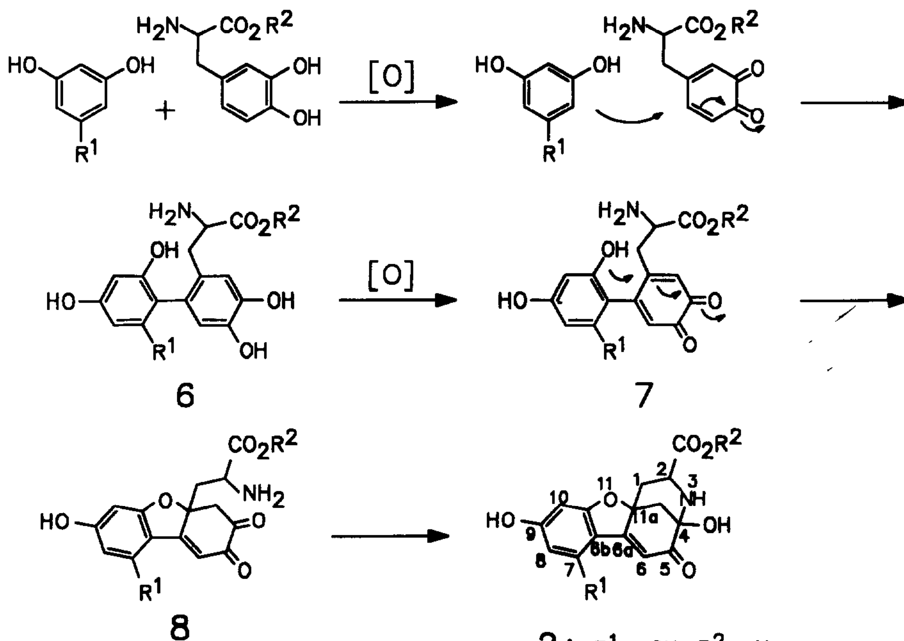
Fig. 1. Effect of phloroglucinol and resorcinol on the oxidation of L-dopa (0.5 mM) catalyzed by tyrosinase (2.2×10^{-2} U/ml) in 0.1 M phosphate buffer, pH 6.8, as determined spectrophotometrically by monitoring dopachrome formation at 475 nm. Curve A: control; curve B: plus 2.5 mM phloroglucinol; curve C: plus 2.5 mM resorcinol.

Reaction of dopa with phloroglucinol was performed on preparative scale using potassium ferricyanide as the oxidizing reagent.[11] Gel filtration chromatography of the reaction mixture on Sephadex G-10, followed by preparative HPLC, resulted in isolation of a yellow compound, negative to ninhydrin, exhibiting an intense chromophore at 377 nm which showed a significant bathochromic shift on treatment with borate at pH 10 and was slowly reduced by sodium borohydride to a colorless species.

The ^{1}H NMR spectrum exhibits in the aromatic region, besides a singlet at δ 6.19 ppm, two doublets (δ 6.01 and 5.91 ppm; J_m=1.6 Hz), consistent with the presence of a monosubstituted pholoroglucinol subunit. The aliphatic region of the spectrum is characterized by an ABX pattern with resonances at δ 1.71, 1.87 and 3.50 ppm, assigned to the $-CH_2CH<$ grouping of the alanyl side chain of a dopa-derived moiety, and a geminal AB system (doublets at δ 2.20 and 2.27 ppm, J=10.6 Hz). This latter feature clearly indicates that formation of the product involved the quaternarization of a substituted aromatic carbon generating a methylene group on the adjacent position.

Of particular value in structural elucidation is also the ^{13}C NMR spectrum showing, inter alia, the singlet of a carbonyl function at δ 194.92 ppm, and two aliphatic singlets at δ 82.37 and 88.57 ppm, indicating direct attachment of heteroatoms.

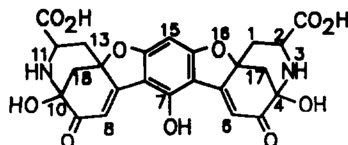
Taken together, all these data could only be accommodated by the tetracyclic structure 2, featuring a tetrahydromethanobenzofuro-[2,3-*d*]azocine ring system. Further support for the presence of a cyclized alanyl side chain is provided by the long range coupling (^4J= 1.3 Hz) between one of the methylene protons of the $-CH_2CH$< group and one of the H-12 protons. Final proof for the structure of the adduct is obtained by long range heteronuclear decoupling and 2D correlation experiments showing that C-11a (seven lines multiplet at δ= 88.57 ppm) is coupled with the methylene protons H-1 and H-12 (^2J= 6 Hz), in addition to the vinylic proton H-6 (^3J= 4 Hz). Decoupling of H-6 results in a triplet, whereas decoupling of H-12 leads to a double-triplet. Furthermore, C-4 (double triplet at δ = 82.37 ppm) is coupled with H-12 (^2J= 6 Hz) and with H-6 (^3J= 5 Hz), as confirmed by decoupling of H-6 (change to a triplet) or H-12 (change to a doublet). The olefinic C-6a at δ 162.08 ppm appears as a



Scheme 1.

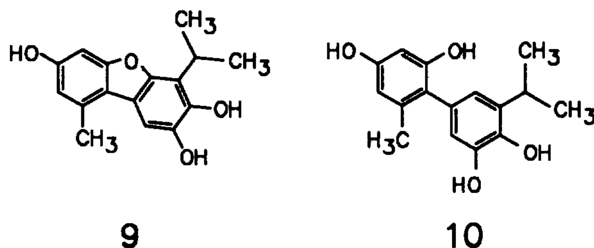
broad triplet like signal in the coupled ^{13}C NMR spectrum. Decoupling of H-12 leads to sharpening and the signal takes the form of a broad doublet, whereas a broad triplet is observed upon decoupling of H-6. Finally, C-6b (quartet like signal at δ 102.03 ppm) is coupled with H-8, H-10 and H-6, as confirmed by decoupling of H-6 (change to a sharp triplet with $^{3}J_{C-6b,H-8}={}^{3}J_{C-6b,H-10}=$ 5 Hz).

Careful analysis of the oxidation mixture revealed the presence of another product, exhibiting an intense absorption maximum at 404 nm. The ^{1}H NMR spectrum is characterized by two aromatic singlets at δ 6.43 and 5.73 ppm with areas in the ratio 2:1, whereas the aliphatic region displays resonances for two equivalent $-CH_2CH<$ groups and two equivalent methylenes. These features, coupled with the presence of 13 signals in the ^{13}C NMR spectrum, led us to formulate the compound as the symmetrical diadduct 3, characterized by an heptacyclic ring system resulting from coupling of phloroglucinol with two dopa derived units. Under the same experimental conditions used for coupling of dopa with phloroglucinol, the reaction of both dopa and dopa methylester with resorcinol was found to proceed similarly leading to the cross coupling products **4** and 5, respectively.



3

Mechanistically, formation of compounds **2**, **4** and **5** can be envisaged as the trapping of the highly reactive *o*-quinone, generated enzymically or chemically from dopa, by 1,4-nucleophilic addition of the activated position of the phenol (phloroglucinol or resorcinol; see Scheme 1). Re-oxidation of the resulting adduct **6** to **7** is followed by intramolecular ring-closure through attack of the phenolic oxygen to the carbon bearing the alanyl side chain. An analogous series of reaction has been suggested by Waiss et al.[12] to account for the formation of the dibenzofuran 9 by autooxidation of 3-isopropyl catechol in the presence of orcinol. In that case, the sequence of the C-C and C-O bond formation was established by allowing the condensation reaction to proceed only partially, which led to the isolation of the adduct **10** as the major product.



The last stage in the formation of compounds **2**, **4** and **5** presumably involves addition of the amino acid side chain of **8** to the spatially more proximal carbonyl group. Although the possibility cannot be ruled out that formation of the aminoacetal function may be reversible at a rate exceeding the time-scale of the usual spectroscopic measurements, its apparent stability is probably due to the possibility of intramolecular closure of a relatively unstrained six-membered ring.

As far as the formation of the diadduct **3** is concerned, the available data do not allow to decide whether attack to the second dopaquinone molecule is brought about by the biphenyl **6** or rather takes place at some further stage of the reaction sequence leading to **2**.

EXPERIMENTAL

UV spectra: Perkin Elmer Lambda 7 spectrophotometer with cell compartment controlled at 25 ± 0.1 °C with circulating water. - ^{1}H NMR (270 MHz) and ^{13}C NMR (67.9 or 100.6 MHz): Bruker AC 270 or WM 400 spectrometer (internal standard: TMS or solvent). - Mass spectra: electron impact (EI-MS): Kratos MS 50 or AEI DS-50, direct inlet, 70 eV; fast atom bombardment (FAB-MS): Kratos MS 50, positive ion mode, matrix: glycerol or *m*-nitrobenzyl alcohol (*m*-NBA). - Analytical and preparative TLC: silica gel F-254 plates (0.25 and 0.50 mm layer thickness, Merck). Paper chromatography: Whatman paper 3MM, using 5% acetic acid as the eluent. The chromatograms were examined by UV irradiation at λ 366 and 254 nm. Ninhydrin (3% acetone solution) was used as spraying reagent. Gel filtration: Sephadex G-10 (Pharmacia). HPLC: Waters pump 6000A; columns: 4x250 mm RP-18 LiChroCART (Merck) or 10x250 mm RP-18 LiChrosorb Hibar (Merck); mobile phase: 0.1 M acetic acid/ methanol 80:20 (v/v); flow rate: 1 ml/min (analytical column) or 6 ml/min (preparative column); detection: Waters 480 UV spectrophotometer (λ 254 nm) and JASCO FP-110 spectrofluorometer (λ_{ex} 366 nm, λ_{em} 440 nm). L-, DL-Dopa and mushroom tyrosinase were purchased from Sigma. DL-dopa methylester hydrochloride was obtained by HCl-methanol esterification of dopa. All other reagents were from Fluka.

Spectrophotometric experiments.
The effect of resorcinol and phloroglucinol on the tyrosinase catalyzed oxidation of L-dopa was followed by monitoring the changes in absorbance at 475 nm. The standard reaction mixture consisted of L-dopa (0.5 mM) and mushroom tyrosinase (2.2×10^{-2} U/ml) in 0.1 M phosphate buffer, pH 6.8. When necessary, aliquots of a freshly prepared solution of phloroglucinol or resorcinol (10 mM) in 0.1 M phosphate buffer, pH 6.8 were added up to 2.5 mM). The tyrosinase unit was defined as the amount of

enzyme required to produce one μmol of dopachrome per min from 0.5 mM L-dopa under the above conditions. In calculating the enzyme units, the molar extinction coefficient of dopachrome at 475 nm was taken[10] as 3600 l $mol^{-1}cm^{-1}$.

Oxidation of dopa by $K_3Fe(CN)_6$ in the presence of resorcinol or phloroglucinol.

To a solution of DL-dopa (2.0 mmol) and resorcinol or phloroglucinol (4.0 mmol) in 0.2 M ammonium bicarbonate buffer (400 ml), pH 8.5, a solution of potassium ferricyanide (8.0 mmol) in water (100 ml) was rapidly added with vigorous stirring. The reaction mixture was allowed to stand under nitrogen atmosphere for 1 h and then extracted twice with ethyl acetate to remove 5,6-dihydroxyindole and the unreacted phenolic compound. The aqueous phase was evaporated to dryness in vacuo at 35 °C and fractionated as follows.

In the case of phloroglucinol, the residue, taken up in water, was chromatographed on a 1.5x100 cm Sephadex G-10 column using 0.03 ammonium bicarbonate as the eluent. Fractions of 10 ml each were collected and monitored by UV. Fractions 18-33, showing absorption maximum around 400 nm, were combined and further purified by preparative HPLC to give 2-carboxy-5-oxo-4,7,9-trihydroxy-2,3,4,5-tetrahydro-1*H*-4,11a-methanobenzofuro[2,3-*d*]azocine (**2**: Rt 13 min, 29 mg). A second yellow band, eluting from the Sephadex column in fractions 10-16, afforded on repeated HPLC fractionation the diadduct **3** (Rt 28 min, 20 mg).

The mixture resulting from reaction with resorcinol was purified by Sephadex G-10 chromatography and HPLC as above to give 2-carboxy-4,9-dihydroxy-5-oxo-2,3,4,5-tetrahydro-1*H*-4,11a-methano-benzofuro[2,3-*d*]azocine (**4**: Rt 14 min, 27 mg).

Oxidation of dopa methylester by $K_3Fe(CN)_6$ in the presence of resorcinol.

DL-dopa methyl ester hydrochloride (2.0 mmol) and resorcinol (4.0 mmol) were treated with potassium ferricyanide (8.0 mmol) under the conditions described above. After standing 1 h under nitrogen, the reaction mixture was repeatedly extracted with ethyl acetate and the combined organic layers were evaporated to dryness. The residue was fractionated by preparative TLC (eluent: ethyl acetate) affording 2-carbomethoxy-4,9-dihydroxy-5-oxo-2,3,4,5-tetrahydro-1*H*-4,11a-methanobenzofuro[2,3-*d*]azocine (**5**: Rf 0.6, 26 mg).

2: UV (H_2O): λ_{max} 252, 377 nm; EI-MS: m/z (%) = 319 (nil, M^+), 257 (0.2, $M^+-CO_2-H_2O$), 232 (2.4, $M^+- C_3H_5NO_2$), 44 (100); HR-MS: m/z = 257.0697 ($C_{14}H_{11}NO_4$, calcd m/z 257.0688), 232.0357 ($C_{12}H_8O_5$, calcd 232.0372); ^1H NMR (270 MHz, DMSO-d_6), δ (ppm): 1.71 and 1.87 (1H, ddd, J=11.8, 4.1, 1.3 Hz, and 1H, dd, J=11.8, 11.8 Hz, H-1a, H-1b), 2.20 and 2.27 (1H, dd, J=10.6, 1.3 Hz and 1H, d, J=10.6 Hz, H-12a, H-12b), 3.50 (1H, dd, J=11.8, 4.1 Hz, H-2), 5.91 (1H, d, J=1.6 Hz, H-8), 6.01 (1H, d, J=1.6 Hz, H-10), 6.19 (1H, s, H-6); ^{13}C NMR (100.6 MHz, DMSO-d_6), δ (ppm): 35.12 (t, C-1), 45.81 (t, C-12), 50.21 (d, C-2), 82.37 (s, C-4), 88.57 (s, C-11a), 90.04 (d, C-8), 96.59 (d, C-10), 102.03 (s, C-6b), 111.90 (d, C-6), 158.40 (s, C-10a), 162.08 (s, C-6a), 165.39, 166.02 (s, s, C-7, C-9), 172.62 (s, C-13), 194.92 (s, C-5).

3: UV (H_2O): λ_{max} 402, 352(sh), 260(sh), 242(sh) nm; ^1H NMR (270 MHz, DMSO-d_6), δ (ppm): 1.81 and 1.91 (2H, dd, J=11.7, 4.0 Hz, and 2H, dd, J=11.7, 11.7 Hz, H-1a, H-13a, H-1b, H-13b), 2.20 and 2.37 (2H, d, J=10.5 Hz and 2H, d, J=10.5 Hz, H-17a, H-18a, H-17b, H-18b), 3.60 (2H, dd, J=11.7, 4.0 Hz, H-2 and H-12), 5.73 (1H, s, H-15), 6.43 (2H, s, H-6 and H-8); ^{13}C NMR (67.9 MHz, DMSO-d_6), δ (ppm): 34.90 (t), 45.11 (t), 50.08 (d), 81.01 (d), 82.41 (s), 88.93 (s), 106.88 (s), 108.45 (d), 159.63 (s), 163.15 (s), 171.66 (s), 172.46 (s), 192.24 (s).

4: UV (H_2O): λ_{max} 255, 299, 375 nm; FAB-MS (glycerol): 304 $(M+H)^+$, 303 (M^+); EI-MS: m/z (%) = 303 (0.6, M^+), 241 (0.5, $M^+-CO_2-H_2O$), 229 (2.5), 216 (5, $M^+-C_3H_5NO_2$), 200 (1), 44 (100); HR-MS: m/z = 303.0744 ($C_{15}H_{13}NO_6$, calcd 303.0743); ^1H NMR (270 MHz, DMSO-d_6), δ (ppm): 1.74 and 1.90 (1H, ddd, J=12.0, 4.0, 1.3 Hz and 1H, dd, J= 12.0, 12.0 Hz, H-1a, H-1b), 2.22 and 2.29 (1H, dd, J= 10.5, 1.3 Hz and 1H, d, J=10.5 Hz, H-12a, H-12b), 3.48 (1H, dd, J=12.0, 4.0 Hz, H-2), 6.32 (1H, s, H-6), 6.44 (1H, d, J=2.0 Hz, H-10), 6.56 (1H, dd, J=8.5, 2.0 Hz, H-8), 7.62 (1H, d, J=8.5, H-7); ^{13}C NMR (67.9 MHz, CF_3COOD), δ (ppm): 33.40 (t), 44.62 (t), 53.28 (d), 87.95 (s), 88.71 (s), 101.23 (d), 111.36 (d), 116.39 (s), 116.62 (d), 129.44 (d), 168.75 (s), 171.09 (s), 172.81 (s), 174.91 (s), 188.87 (s).

5: UV (MeOH): λ_{max} 259, 300, 371 nm; FAB-MS (*m*-NBA): m/z = 340 $(M+Na)^+$; EI-MS: m/z (%) = 317 (27, M^+), 289 (8), 288 (18, M^+-CHO), 258 (11, M^+-COOMe), 229 (9, M^+-COOMe-CHO), 216 (13, $M^+-C_4H_7NO_2$), 188 (20), 187 (100, $M^+-C_4H_7NO_2$-CHO), 161 (7), 45 (15), 44 (8), 43 (22), 32 (7), 31 (10); HR-MS: m/z 317.0902 ($C_{16}H_{15}NO_6$, calcd 317.0899);^1H NMR (270 MHz, DMSO-d_6), δ (ppm): 1.75 and 1.94 (1H, ddd, J= 10.2, 4.1, 1.3 Hz and 1H, dd, J=12.1, 12.1 Hz, H-1a, H-1b), 2.23 and 2.30 (1H, dd, J=10.6, 1.3 Hz and 1H, d, J=10.6 Hz, H-12a, H-12b), 3.60 (1H, dd, J=12.1, 4.1 Hz, H-2), 3.60 (3H, s, OCH_3), 6.34 (1H, s, H-6), 6.54 (1H, d, J=2.0 Hz, H-10), 6.56 (1H, dd, J=8.5, 2.0 Hz, H-8), 7.62 (1H, d, J=8.5 Hz, H-7); ^{13}C NMR (DMSO-d_6), δ (ppm): 34.39 (t), 45.60 (t), 50.06 (d), 51.81 (q), 82.17 (s), 88.26 (s), 97.76 (d), 111.24 (d), 112.10 (d), 112.61 (s), 126.01 (d), 162.62 (s), 164.40 (s), 165.44 (s), 171.37 (s), 195.01 (s).

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REFERENCES

1. Peter, M.G. *Angew. Chem.* **1989**, *101*, 572-587; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 555-570.
2. Prota, G. *Med. Res. Rev.* **1988**, *8*, 525-556.
3. Swan, G.A. *Fortschr. Chem. Org. Naturst.* **1974**, *31*, 521-596.
4. Omote, Y.; Komatsu, T. *J. Chem. Soc. Chem. Comm.* **1974**, 792-793.
5. Omote, Y.; Hirama, T.; Komatsu, T. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 1957-1960 .
6. Omote, Y.; Tomotake, A.; Kashima, C. *J. Chem. Res.* **1987**, 10-11.
7. Peter, M.G.; Grittke, U., Grün, L., Schäfer, D. in *Endocrinological Frontiers in Physiological Insect Ecology*; Senhal, F.; Zabza, A.; Denlinger, D.L. Eds.; Wroclaw Technical University Press: Wroclaw, 1988; pp. 519-530.
8. Miessner, M.; Cresecenzi, O.; Napolitano, A.; Prota, G.; Andersen, S.O.; Peter, M.G. in preparation.
9. Wong, T.C.; Luh, B.S.; Whitaker, J.R. *Plant Physiol.* **1971**, *48*, 24-30.
10. Mason, H.S. *J. Biol. Chem.* **1948**, *172*, 83-99.
11. Sobotka, H.; Barsel, N.; Chanley, J.D. *Fortschr. Chem. Org. Naturst.* **1957**, *14*, p. 220.
12. Waiss, A.C.; Kuhnle, J.A.; Windle, J.J.; Wierseman, A.K. *Tetrahedron Lett.* **1966**, *50*, 6251-6255.