Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, No. 3, pp. 307–312, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00
© 1990 Pergamon Press plc

Short Communication

Improved preparation and structural conformation of the fluorescence labelling reagents 1,2-diamino-4,5-dimethoxybenzene and 3,4-dihydro-6,7-dimethoxy-4-methyl-3-oxo-quinoxaline-2-carbonyl chloride

K.J. DAVE,* C.M. RILEY,* D. VANDER VELDE† and J.F. STOBAUGH*‡

*Departments of * Pharmaceutical Chemistry and † Medicinal Chemistry, University of Kansas, Lawrence, KS 66045, USA*

Keywords: *Liquid chromatography; fluorescence labels; 1,2-diamino-4,5-dimethoxybenzene; 3,4-dihydro-6,7-dimethoxy-4-methyl-3-oxo-quinoxaline-2-carbonyl chloride; nuclear Overhauser effect; synthesis.*

Introduction

Fluorescence detection, when used in combination with high-performance liquid chromatography (HPLC), forms the basis for some of the most sensitive methods in biopharmaceutical analysis. Accordingly, numerous fluorescence labelling reagents have been developed, that are applicable in the trace determination of analytes bearing functional groups amenable to covalent chemical modification [1].

Interest in these laboratories has recently been focused on the determination of several substances that possess a secondary alcohol functional group as a derivatization site. Review of the literature revealed, that numerous alcohol reagents have been reported [2–12], and that a recent publication detailed the preparation and use of 3,4-dihydro-6,7-dimethoxy-4-methyl-3-oxo-quinoxaline-2-carbonyl chloride (DMEQ–COCl) as a primary or secondary alcohol reagent [13], that allowed mass-detection in the low fmol range by conventional HPLC. Additionally, conversion of DMEQ–COCl into the corresponding azide (DMEQ–CON_3), resulted in a reagent with a reactivity that is suitable for the derivatization of tertiary alcohols [14].

Interestingly, 1,2-diamino-3,4-dimethoxybenzene (DDB) (Figs 1 and 2), a fluorogenic reagent for aldehydes and α-dicarbonyl compounds such as glyoxals and α-ketoacids [15–23], is required for the preparation of both DMEQ–COCl, DMEQ–CON_3, and the carboxylic acid labelling reagent, 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone [24, 25]. Thus, DDB, which itself is useful for the trace determination of several classes of chemical substances, is a key synthetic intermediate in the preparation of three other fluorescence labelling reagents.

Due to the excellent detection limits reported for primary and secondary alcohols derivatized by reaction with DMEQ–COCl [13], this reagent was selected for use in these laboratories. However, since neither DMEQ–COCl nor the precursor DDB was commercially available, the preparation of these compounds was initially carried out by procedures published by the innovators [13]. Unfortunately, several steps of the published route were difficult to reproduce or resulted in complex mixtures that were difficult to resolve. In addition, during this investigation a doubt arose as to whether *N*-methylation or *O*-methylation of quinoxalinone-2-carboxylic acid had occurred when synthesized by the previously reported method (Fig. 1, Path B) [26, 27]. Therefore, an alternative method was sought for the preparation of the required reagent.

‡Author to whom correspondence should be addressed.

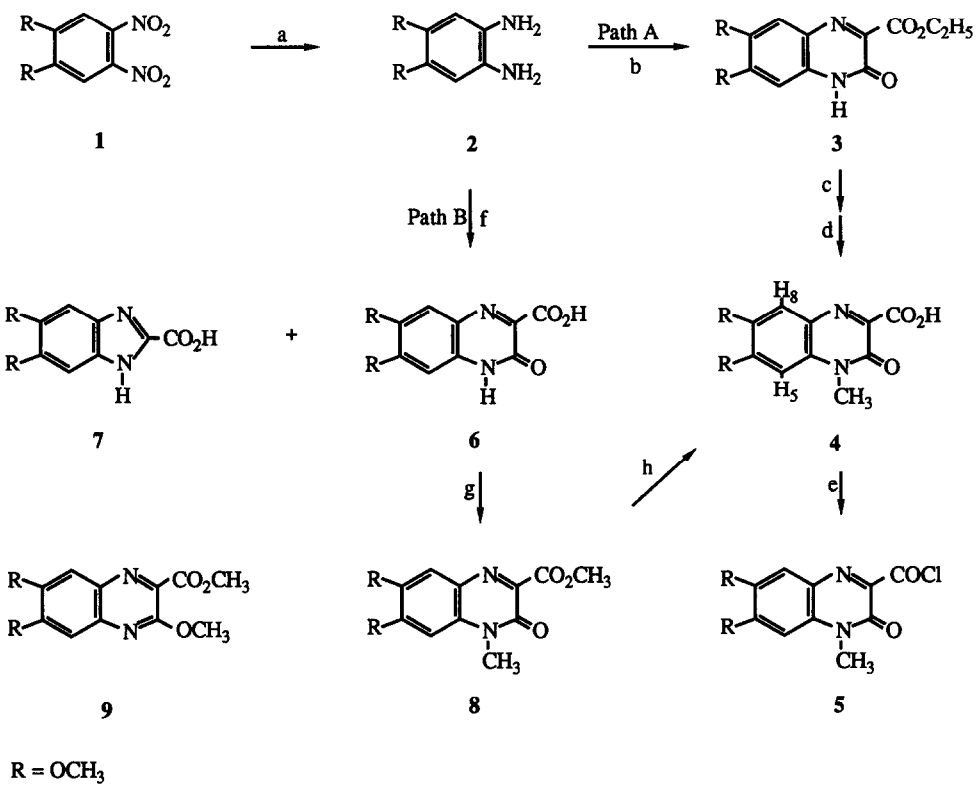

Figure 1
Synthetic route for preparation of the reagents DDB and DMEQ–COCl. (a) PtO_2, H_2; (b) $CO(CO_2C_2H_5)_2$, Δ; (c) $(CH_3)_2SO_4$, Δ; (d) NaOH; (e) $SOCl_2$, Δ; (f) $CO(CO_2H)_2$, HCl, Δ; (g) Ch_2N_2; (h) NaOH.

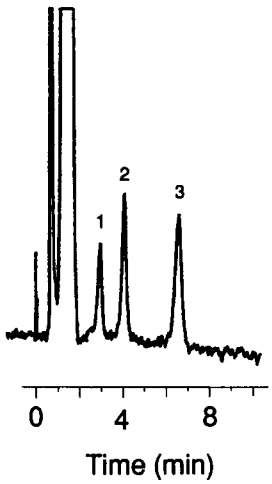

Figure 2
A chromatogram of a mixture of alcohols derivatized with DMEQ–COCl. Each peak represents the equivalent of 1 pmol injected. Peak identity: (1) benzyl alcohol, (2) cyclohexanol and (3) *n*-hexanol. See the Experimental section for chromatographic conditions and parameters.

As a result of this research, a simple method for the preparation of DDB and DMEC–COCl has been established, the structure of the resulting reagent has been unambiguously determined, and the utility of DMEQ–COCl in the derivatization of primary and secondary alcohols has been confirmed.

Experimental

Apparatus and materials

Melting points (uncorrected) were determined on an electrothermal melting point device. Magnetic resonance spectra (^1H) were obtained by using Varian Associates model EM 360 (60 MHz), FT 80A (80 MHz) and XL 300 (300 MHz) spectrometers and a Bruker model AM 500 (500 MHz) spectrometer. Tetramethylsilane (TMS) and 2,2-dimethyl-2-silapentane-5-sulphonate sodium salt (DSS) were used as internal references for organic and aqueous samples, respectively. Infrared absorption spectra were recorded with a Beckman Acculab spectrophotometer. Mass spectra were obtained with a Nermag R-10-10 quadrupole instrument. Fluorescence spectra were obtained with a Perkin–Elmer Model 650-40 spectrofluorimeter interfaced to a Model 3600 data station. Elemental analyses

were performed by the Department of Medicinal Chemistry in the University of Kansas utilizing a Hewlett–Packard model 185B CHN analyzer. Catalytic hydrogenation was accomplished with a Parr-shaker apparatus. Thin-layer chromatography (TLC) was performed on plastic backed silica gel 60 F_{254} plates, 0.2 mm thickness (E. Merck). A modular HPLC system was assembled from an LKB 2150 pump, a Rheodyne 7125 fixed loop (20 μl) injector and a St. John Associates Fluoro-Tec filter fluorimeter, which was equipped with a mercury vapour lamp as the excitation source, a Corning 7-54 primary filter, a 450 nm cut-off secondary filter and a 9-μl flow cell. The HPLC column (150 × 4.6 mm, i.d.) was packed with Hypersil MOS (5 μm) using an upward slurry procedure. The chromatography solvents and synthetic starting materials were of the highest purity available from commercial sources and were used as received.

Synthetic preparations

4,5-Dinitro-1,2-dimethoxybenzene (1). The product was prepared by nitration of 1,2-dimethoxybenzene according to an established procedure [28]. Recrystallization of the crude residue from ethanol provided the desired product: 89% yield; m.p. 130°C (ref. 28, m.p. 129.5°C); ^{1}H NMR (60 MHz, $CDCl_3$) δ 4.20 (s, 6 H), 7.48 (s, 2 H).

4,5-Diamino-1,2-dimethoxybenzene dihydrochloride (2, DDB). A solution of **1** (1.0 g, 4.4 mmol) in ethyl acetate–absolute ethanol (9:1; 100 ml) was hydrogenated (50 psi) over platinum oxide (0.1 g) for 12 h. The reaction mixture was then filtered through a Celite pad into HCl-saturated ethyl acetate–absolute ethanol (9:1; 20 ml). The resulting precipitate was recrystallized from absolute ethanol. This solid was dried overnight in a vacuum oven (40°C) to provide the required product: 0.84 g, 71%; m.p. 249–250°C (dec); single spot TLC (methanol, R_f = 0.65); ^{1}H NMR (80 MHz, $CDCl_3$) δ 3.76 (s, 6 H), 6.87 (s, 2 H); MS (EI), *m/z* 168 (M^+, free base). Anal. Calcd for $C_8H_{12}N_2O_2.2HCl$: C, 39.83; H, 5.81; N, 11.62. Found: C, 40.10; H, 6.00; N, 11.80.

Ethyl-3,4-dihydro-6,7-dimethoxy-3-oxo-quinoxaline-2-carboxylate (3). Diethylketomalonate (1 g, 5.75 mmol) was dissolved in absolute ethanol (25 ml) and added to a cool (4°C) absolute ethanolic solution (25 ml) of **2** (1.1 g, 4.4 mmol). The reaction mixture was warmed to reflux for 15 min. After cooling at 4°C for 3 h, a solid formed that was recovered by filtration. The crude product was purified by recrystallization from absolute ethanol: 71%; m.p. 249–250°C (ref. 29, m.p. 251–252°C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (t, 3 H), 3.98 (q, 2 H), 7.01 (s, 1 H), 7.38 (s, 1 H); MS (EI), *m/z* 278 (M^+).

3,4-Dihydro-6,7-dimethoxy-4-methol-3-oxo-quinoxaline-2-carboxylic acid (4). To a solution of **3** (2.0 g, 7.2 mmol), prepared by dissolution in warm acetone (40°C), were added potassium carbonate (3.5 g, 25.2 mmol) and dimethylsulphate (0.67 ml, 6.7 mmol). The reaction was maintained at 40°C for 3 h, after which the solvent was evaporated and the residue dissolved in a solution of sodium hydroxide (1 M, 100 ml). After standing at ambient temperature for approximately 1 h, the mixture was filtered, washed three times with ethyl acetate (150 ml), and the aqueous layer recovered and neutralized with dilute hydrochloric acid. The resulting precipitate was collected and recrystallized from 1,4-dioxane–water (4:1) to provide a solid that was dried overnight in a vacuum oven (70°C): 0.86 g, 45%; m.p. 234–235°C (ref. 29, m.p. 233–235°C; ref. 13, m.p. 222°C); ^{1}H NMR (500 MHz D_2O–NaOD) δ 3.89 (s, 3 H), 3.98 (s, 3 H), 4.11 (s, 3 H), 6.77 (s, 1 H), 7.65 (s, 1 H); MS (EI) *m/z* 264 (M^+).

3,4-Dihydro-6,7-dimethoxy-4-methyl-3-oxo-quinoxaline-2-carbonyl chloride (5). DMEQ–COCl). The carboxylic acid **5** was dissolved in freshly distilled thionyl chloride (10 ml), refluxed for 1 h, then cooled to ambient temperature. Upon addition of petroleum ether (25 ml, b.p. 35–60°C) a precipitate formed that was recrystallized from benzene–petroleum either (9:1) to provide an orange solid which was dried overnight under vacuum at ambient temperature: 0.37 g, 72%; m.p. 220°C (ref. 13, m.p. 261°C).

Attempted preparation of 3,4-Dihydro-6,7-dimethoxy-3-oxo-quinoxaline-2-carboxylic acid (6). Disodium ketomalonic acid monohydrate (0.47 g, 2.4 mmol) and **2** (0.5 g, 2.1 mmol) were dissolved in hydrochloric acid (0.5 M, 20 ml) and the reaction mixture was refluxed for 2 h. Upon cooling, a precipitate formed,

which was recovered by filtration and washed with water. After recrystallization [1,4-dioxane–water (9:1)], TLC characterization (ethyl acetate–hexane (9:1) indicated the presence of two compounds (R_f 0.12, 0.17). Mass spectral characterization of the mixture revealed that the desired product had formed, but significant quantities of 5,6-dimethoxybenzimidazole-2-carboxylic acid (**8**) had apparently formed as an additional product: (EI), *m/z* (relative intensity), 250 (2, M^+, component 1), 222 (100, M^+, component 2); MS (CI, NH_3), *m/z* (relative intensity) 251 (2, M + 1, component 1), 223 (100, M + 1, component 2).

Nuclear Overhauser effect (nOe)

The Bruker AM-500 magnetic resonance spectrometer was used to perform one-dimensional nOe-difference experiments. Three spectra were obtained in which the methyl signals were individually irradiated and a suitable control spectrum was generated by irradiating off-resonance with respect to the region of interest. The nOe effects were determined by computer subtraction of the blank from each of the methyl-irradiated spectra.

Derivatization

A test solution containing benzyl alcohol, cyclohexanol and *n*-hexanol (5.0×10^{-5} M) was prepared in benzene. To 500 μl of this test solution contained in an amber screw-capped vial was added a 500-μl aliquot of DMEQ–COCl (3.0×10^{-3} M) dissolved in benzene. The vial was tightly closed and heated at 100°C for 40 min. After cooling, a 20-μl aliquot of the reaction mixture was diluted with 2.0 ml of methanol and the resulting solution was analysed by HPLC using water–methanol (40:60, v/v) as the mobile phase.

Results and Discussion

Reagent synthesis

The previously described route for the preparation of DDB (**2**) [15] and DMEQ–COCl (**5**) [13], as shown by Path B of Fig. 1, was initially attempted with limited success. Accordingly, the following alterations were made, which resulted in an improved approach for the preparation of DDB and DMEQ–COCl.

Rather than by reducing **1** by a dissolving metal reaction (Fe/HCl), it was found that **2** was readily formed by catalytic hydrogenation over platinum oxide and was isolated as the stable dihydrochloride salt, thus avoiding the need for extraction of the oxidatively labile free base and the use of benzene as a solvent. Quinoxalinone ring formation was first attempted by condensation of α-ketomalonic acid with DDB in acidic media [13]. After recrystallization of the isolated solid, two products were observed by TLC in a ratio of approximatey 4:1. Mass spectral chracterization of this mixture suggested the presence of two substances whose molecular weights were 222 (major product) and 250 (minor product), thus indicating that while the required product (Fig. 1, **6**) was formed, it appeared to be contaminated with large quantities of 5,6-dimethoxy-benzimidazole-2-carboxylic acid, **7**. Such results have previously been noted by others with similar reactants when the reaction was conducted in basic media [30]. However, by simply using diethyl α-ketomalonate instead of the acid (Fig. 1, Path A), and conducting the reaction in absolute ethanol [29], the product , ethyl 3,4-dihydro-6,7-dimethoxy-3-oxo-quinoxaline-2-carboxylate (**3**) was formed in high yield and purity.

With the ester **3** easily obtainable, all that remained for the preparation of DMEQ–COCl (Fig. 1, **5**) was methylation of the quinoxalinone ring system and transformation of the carboxylic acid ester to the corresponding acid chloride. Alkylation of **3** was accomplished by reaction with dimethyl sulphate in acetone in the presence of potassium carbonate [29] and, without isolation of the product, hydrolysis of the reaction mixture provided **4**. Finally, the corresponding carboxylic acid chloride was obtained by treatment of **4** with thionyl chloride.

When the fluorescence spectrum of **4** was obtained in methanolic solution, excitation (396 nm) and emission (470 nm) maxima were obtained, which were virtually identical to those previously reported by Iwata *et al.* [13]. Thus, it appeared that the improved synthetic route did result in the formation of the same product. However, of concern was the observation that **4** exhibited a melting point of 234–235°C, which significantly differed (+10°C) from the Iwata *et al.* [13] preparation. Ahmad *et al.* [29] have previously prepared **4** by methylation of **3** with dimethyl sulphate with subsequent hydrolysis and reported the resulting carboxylic acid to melt at 233–235°C, which agrees with the results presented here.

In general, 2-substituted quinoxalin-3-ones have been found to undergo both *N*-and *O*-alkylation with ethereal diazomethane, while treatment of the quinoxalinone anion with either dimethyl sulphate or methyl iodide results exclusively in *N*-methylation [26, 31]. Based on this information and the discrepancy in the melting point of **4**, there was concern as to whether Iwata *et al.* [13] had formed the methylated quinoxalinone ester **8** or the isomeric quinoxaline derivative **9** when **6** was treated with ethereal diazomethane. If **9** had resulted, then the subsequent transformations would have produced 3-methoxyquinoxaline-2-carboxylic acid chloride rather than the claimed DMEQ–COCl. In either case, a sensitive and useful reagent had been prepared [13]. However, it is of scientific interest to assign the correct structure to this new fluorescent labelling agent.

Structure elucidation

The determination of whether *N*- or *O*-methylation had occurred in the preparation of product **4** cannot be answered unambiguously from infrared, ultraviolet or ^{1}H NMR spectra. However, magnetic resonance experiments, in which the spin-lattice relaxation times T_1 of individual resonances are determined and used in combination with the observation of an intramolecular nuclear Overhauser effect (nOe), were able to clarify this issue [32].

The spin-lattice relaxation time (T_1) is the rate constant for a particular saturated nucleus (all spin states are equally populated) to achieve the natural Boltzmann population distribution once the exciting irradiation source is removed. While numerous physical mechanisms contribute to the relaxation process, of interest in the present problem is the contribution due to dipole–dipole interactions between nearby nuclei. The close proximity of another nucleus enhances the relaxation process. In the present problem, if **4** was produced as a result of *N*-methylation (Fig. 1), the T_1 value observed for H_8 would be expected to be significantly larger than the H_5 relaxation time. Conversely, if *O*-methylation had resulted, approximately equivalent T_1 values would be expected for H_5 and H_8 (refer to the ring system and substituents of **9** in Fig. 1). When T_1 values were determined for the various proton resonances of **4** prepared by the present method, the values summarized in Table 1(A) resulted. These data indicate that the single proton resonances observed at δ 6.77 and δ 7.64 exhibit significantly different spin-lattice relaxation times, which suggests that the structural representation of **4** shown in Fig. 1 is correct. Additionally, this information indicates that the resonance observed at δ 6.77 can be assigned to H_5, since this proton rather than H_8 is in an environment that allows enhanced relaxation due to dipole–dipole interaction.

Table 1
^{1}H NMR characterization of product **4**
(A) Results from the determination of spin-lattice relaxation times T_1

δ	T_1(s)
3.89	1.79
3.98	1.24
4.11	1.21
6.77 (H_5)	1.71
7.64 (H_8)	3.93

(B) Results from the nOe experiments

Signal irradiated (δ)	Signal enhanced (δ)	% Enhancement
3.89	6.78 (H_5)	17
3.99	7.64 (H_8)	38
4.11	6.78 (H_5)	23

In a homonuclear ^{1}H nOe experiment, nucleus A is saturated by strong irradiation while the resonance intensity of nucleus B is simultaneously monitored. If B is spatially located with respect to A such that an effective dipole–dipole interaction can occur, a significant enhancement of the B resonance [32] often results. In the present case, the observation of the nOe effect allowed differentiation between the ring substitution isomers such as **4** versus **9**. If the structural representation of **4** in Fig. 1 is correct, then H_5 will exhibit the strongest resonance enhancement when two of the three neighbouring methyl substituents are irradiated. However, if **4** actually possesses the ring substitution pattern as in **9**, then irradiation of only one methyl substituent will result in H_5 displaying the dominant resonance enhancement. When the experiment was conducted, the proton resonance at δ 6.775 (Table 1B) displayed the strongest signal enhancement when two of the neighbouring methyl substituents were irradiated. These nOe results are in agreement with the conclusions previously drawn from the spin-lattice relaxation measurements and thus when considered together provide conclusive evidence that **4** was correctly assigned as the quinoxalinone as

shown in Fig. 1. Finally, based on the similarity of fluorescence excitation and emission properties of **4**, one may reasonably conclude that the earlier work of Iwata *et al.* [13] did result in the formation of an *N*-methylated quinoxalinone.

Derivatization

To confirm the suitability of DMEQ–COCl as a fluorescence labelling reagent for the trace determination of primary and secondary alcohols, a test mixture consisting of *n*-hexanol, benzyl alcohol and cyclohexanol was derivatized. The conditions previously suggested by Iwata *et al.* [13] were used. After completion of the reaction, the resulting derivatives were separated by RP–HPLC to produce the chromatogram shown in Fig. 2. It should be noted that the detector that was used to generate the chromatogram in Fig. 2 was a relatively simple filter fluorimeter. Nevertheless a good *S/N* ratio (10:1 minimum) resulted for these test alcohols at the 1 pmol on-column level.

Conclusions

As a result of this investigation, improved synthetic methodology has been established for the preparation of DDB and DMEQ–COCl. This straightforward method for the preparation of DDB is particularly useful as this reagent is necessary for the synthesis of three other fluorescent labelling reagents. The nOe experiments allowed the unambiguous structural assignment for DMEQ–COCl, which was found to be in agreement with that previously assigned [13]. In a brief test of the applicability of the reagent, its ability to label a mixture of alcohols was confirmed. Thus it appears that DMEQ–COCl is a reagent which can be very useful in the trace determination of analytes bearing a primary or secondary alcohol functional group.

Acknowledgements — The authors would like to thank Hoffmann-LaRoche Inc., for partial funding of this research and Dr N. Meltzer for his interest.

References

[1] H. Lingeman, W.J.M. Underberg, A. Takadate and A. Hulshoff, *J. Liq. Chromatogr.* **8**, 789–874 (1985).
[2] R. Wintersteiger, G. Wenninger-Weinzierl and W. Pacha, *J. Chromatogr.* **237**, 399–406 (1982).
[3] J. Goto, S. Komatsu, N. Goto and T. Nambara, *Chem. Pharm. Bull.* **29**, 899–901 (1981).
[4] J. Goto, N. Goto and T. Nambara, *Chem. Pharm. Bull.* **30**, 4597–4599 (1982).
[5] J. Goto, N. Goto, F. Shamsa, M. Saito, S. Komatsu, K. Suzaki and T. Nambara, *Anal. Chim. Acta* **147**, 397–400 (1983).
[6] R. Wintersteiger, G. Gamse and W. Pacha, *Fresenius' Z. Anal. Chem.* **312**, 455–461 (1982).
[7] O.S. Wolfbeis and H. Marhold, *Monatsh. Chem.* **114**, 599–604 (1983).
[8] A. Takadate, M. Iwai, H. Fujino, K. Tahara and S. Goya, *Yakugaku Zasshi* **103**, 962–966 (1983).
[9] C. Hamada, M. Iwasaki, N. Kuroda and Y. Ohkura, *J. Chromatogr.* **341**, 426–431 (1985).
[10] K.E. Karlsson, D. Wiesler, M. Alasandro and M. Novotny, *Anal. Chem.* **57**, 229–234 (1985).
[11] A. Takadate, T. Tahara, H. Fujino and S. Goya, *Chem. Pharm. Bull.* **30**, 4120–4125 (1982).
[12] A. Takadate, M. Irikura, T. Suehiro, H. Fujino and S. Goya, *Chem. Pharm. Bull.* **33**, 1164–1169 (1985).
[13] T. Iwata, M. Yamaguchi, S. Hara and M. Nakamura, *J. Chromatogr.* **362**, 209–216 (1986).
[14] M. Yamaguchi, T. Iwata, M. Nakamura and Y. Ohkura, *Anal. Chim. Acta* **193**, 209–217 (1987).
[15] M. Nakamura, M. Toda, H. Saito and Y. Ohkura, *Anal. Chim. Acta* **134**, 39–45 (1982).
[16] M. Nakamura, M. Toda, K. Mihashi, M. Yamaguchi and Y. Ohkura, *Chem. Pharm. Bull.* **31**, 2910–2912 (1983).
[17] S. Hara, Y. Takemori, M. Yamaguchi, M. Nakamura and Y. Ohkura, *J. Chromatogr.* **344**, 33–39 (1985).
[18] S. Hara, Y. Takemori, T. Iwata, M. Yamaguchi, M. Nakamura and Y. Ohkura, *Anal. Chim. Acta* **172**, 167–173 (1985).
[19] S. Hara, M. Yamaguchi, M. Nakamura and Y. Ohkura, *Chem. Pharm. Bull.* **33**, 3493–3498 (1985).
[20] T. Iwata, S. Hara, M. Yamaguchi, M. Nakamura and Y. Ohkura, *Chem. Pharm. Bull.* **33**, 3499–3502 (1985).
[21] T. Iwata, M. Yamaguchi, S. Hara, M. Nakamura and Y. Ohkura, *J. Chromatogr.* **344**, 351–355 (1985).
[22] T. Yoshitake, S. Hara, M. Yamaguchi and M. Nakamura, *J. Chromatogr.* **489**, 364–370 (1989).
[23] M. Kai, J. Ishida and Y. Ohkura, *J. Chromatogr.* **430**, 271–278 (1988).
[24] M. Yamaguchi, S. Hara, R. Matsunaga, M. Nakamura and Y. Ohkura, *J. Chromatogr.* **346**, 227–236 (1985).
[25] M. Yamaguchi, K. Fukuda, S. Hara and M. Nakamura, *J. Chromatogr.* **380**, 257–265 (1986).
[26] G.W.H. Cheeseman, *J. Chem. Soc.* 1804–1809 (1955).
[27] G.W.H. Cheeseman, *J. Chem. Soc.* 1246–1251 (1961).
[28] N.L. Drake, H.D. Anspon, J.D. Draper, S.T. Haywood, J. van Hook, S. Melamed, R.M. Peck, J. Starling Jr, E.W. Walton and A. Whiton, *J. Am. Chem. Soc.* **68**, 1536–1543 (1946).
[29] Y. Ahmad, M.S. Habib, M. Iqbal and M.I. Qureshi, *J. Chem. Soc.* 4053–4057 (1964).
[30] E.C. Taylor and M.J. Thompson, *J. Org. Chem.* **26**, 3511–3513 (1961).
[31] G.W.H. Cheeseman and R.F. Cookson, in *The Chemistry of Heterocyclic Compounds* (A. Weissberger and E.C. Taylor, Eds), Vol. 35, p. 88. Wiley, New York (1979).
[32] G.E. Bachers and T. Schaefer, *Chem. Rev.* **71**, 617–626 (1971).

[*Received for review 28 August 1989*]