

COOKSON-TYPE REAGENTS: APPLICATION TO THE DETERMINATION OF 7-DEHYDROCHOLESTEROL IN HUMAN SKIN SURFACETOMOYUKI OE, TATSUHIITO MIZUGUCHI and KAZUTAKE SHIMADA

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Abstract - One of the Cookson-type reagents, 4-(1-anthryl)-1,2,4-triazoline-3,5-dione, has been applied to the determination of 7-dehydrocholesterol in human skin surface. The extract from human skin surface was derivatized with the reagent and the obtained adduct was selectively detected using a UV detector.

Key words Cookson-type reagent, 4-(1-anthryl)-1,2,4-triazoline-3,5-dione, high performance liquid chromatography, 7-dehydrocholesterol, human skin surface

7-Dehydrocholesterol (7-DHC) is present in human skin and is converted photochemically into vitamin D₃ via pre-vitamin D₃ [1]. The interest has recently been focused on the correlation between age and the 7-DHC concentration level in human skin [2]. Iwata et al. converted 7-DHC into the fluorescent derivative and determined with high performance liquid chromatography (HPLC). The method is sensitive but it takes 60 min at 120°C to convert 7-DHC into the desired derivative [3]. Recently, Moody et al. reported the determination of 7-DHC by HPLC with electrochemical detection [4]. But the detector requires the long warming-up time to get the stable base line on the chromatogram, because the high applied potential is necessary to get the high sensitivity. The Cookson reagent, 4-phenyl-1,2,4-triazoline-3,5-dione, has been used as a protecting group for the conjugated diene moiety in the synthesis of vitamin D related compounds [5]. In previous papers, the syntheses of Cookson-type reagents having a chromophore, fluorophore or electrophore at the 4-position have been reported for high performance liquid chromatographic measurements of conjugated dienes [6,7]. These reagents gave stable Diels-Alder adducts quantitatively under mild conditions within a short period of time. The present work deals with the application of one of the Cookson-type reagents, 4-(1-anthryl)-1,2,4-triazoline-3,5-dione (I) (Fig. 1), to the determination of 7-DHC in human skin surface.

EXPERIMENTAL

Chemicals and apparatus 7-DHC and β -sitosterol were obtained from Wako (Osaka, Japan) and Funakoshi (Tokyo, Japan), respectively. The HPLC measurements were carried out on a Shimadzu LC-6A chromatograph equipped with a Shimadzu SPD-6AV UV detector (253 nm) (Shimadzu, Kyoto, Japan). A YMC-GEL C₈-120-S5 column (YMC, Kyoto) (15 x 0.46 cm i.d., 5 μ m) was used under ambient temperature at a flow rate of 1.0 ml/min. Silica gel 60 (70-230 mesh: E. Merck, Darmstadt, F. R. Germany) was used for column chromatography.

Synthesis of 7-dehydro- β -sitosterol β -Sitosterol was converted to 7-dehydro derivative as usual way [8]. The obtained 7-dehydro- β -sitosterol was used as an internal standard (IS). The structure of which was confirmed by proton nuclear magnetic resonance and high-resolution mass spectra.

Determination of 7-DHC in human skin surface Male human (23 years old) skin surface (11.34 cm²; forearm) was extracted with hexane-EtOH (1:1; three 5 ml portions; 5 min for each extraction) [3] and IS (50 ng) was added to the extract. After concentration of the solvent to 0.5 ml, the sample was filtered through a glass filter (G-2). The filtrate was evaporated under a stream of nitrogen and the residue obtained was dissolved in CH₂Cl₂ (1 ml).

The EtOAc solution of I (about 36.3 μ g) was added to the above sample and kept at 4°C for 10 min. The solution was subjected to silica gel column (6 x 0.6 cm i.d.) and the eluate with EtOAc-CH₂Cl₂ (11:9) was subjected to HPLC.

RESULTS AND DISCUSSION

Of the Cookson-type reagents examined, I has been chosen as a derivatization reagent for the determination of 7-DHC in human skin surface [7]. The adduct (II) gave a single peak on the chromatogram from reversed-phase HPLC and was highly sensitive to UV detector [detection limit 60 fmol (signal to noise ratio=5)]. Human skin surface was extracted with hexane-ethanol and the IS was added to the extract. After the derivatization with I, the reaction mixture was filtered through the small silica gel column. The recovery rate of II (50 ng) from the column was $93.4 \pm 3.0\%$ ($n=6$; mean \pm S.D.). The eluate was subjected to HPLC and the typical chromatogram was shown in Fig. 2. The concentration of 7-DHC in forearm of male human skin surface was determined as 8.1 ng/cm². Peak was assigned by comparison of the chromatographic behavior with that of the authentic sample using other solvent system [acetonitrile-methanol-water (2:3:1), t_R 20.4 min]. The proposed method is highly sensitive and selective to determine the compound having the diene moiety. Studies into the application of this reaction to the determination of other vitamin D₃ related compounds are currently in progress in this laboratory.

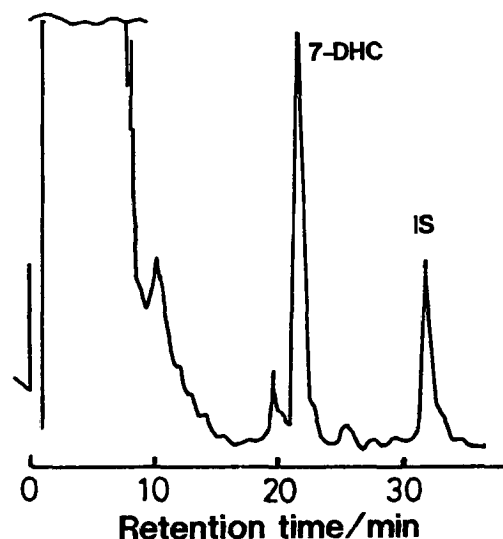
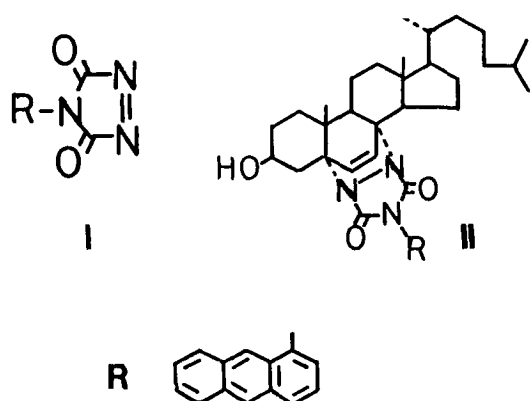


Fig. 1. Structures of Cookson-type reagent (I) and its adduct (II) with 7-DHC

Fig. 2. Chromatogram of the adduct (II) of 7-DHC in human skin surface with I. Mobile phase: MeOH-H₂O (5:1)

REFERENCES

1. K. Shimada and N. Kobayashi, *Trends Anal. Chem.*, **10**, 103 (1991).
2. J. MacLaughlin and M. F. Holick, *J. Clin. Invest.*, **76**, 1536 (1985).
3. T. Iwata, H. Hanazono, M. Yamaguchi, M. Nakamura and Y. Ohkura, *Anal. Sci.*, **5**, 671 (1989).
4. J. P. Moody, C. A. Humphries, S. M. Allan and C. R. Paterson, *J. Chromatogr.*, **530**, 19 (1990).
5. D. H. R. Barton, T. Shioiri and D. A. Widdowson, *J. Chem. Soc., Chem. Commun.*, **1970**, 939.
6. K. Shimada and T. Oe, *Anal. Sci.*, **6**, 461 (1990).
7. K. Shimada, T. Oe and T. Mizuguchi, *Analyst*, in press.
8. N. Kobayashi, A. Hisada and K. Shimada, *Chem. Ind. (London)*, **1990**, 803.