Interaction between dehydroacetic acid sodium salt and formaldehyde: Structural identification of the product

C. A. BENASSI, A. BETTERO, P. MANZINI,
A. SEMENZATO, and P. TRALDI,* *Department of Pharmaceutical Sciences, University of Padova, Via Marzolo 5, I 35131 Padova, and C.N.R.,* *Corso Stati Uniti 4, I 35100 Padova, Italy.*

Synopsis

The structural identification of the product formed by the interaction between dehydroacetic acid sodium salt (Prevan®) and formaldehyde, either free or released from some other preservatives commonly employed in the cosmetic field, has been obtained by elemental analysis, ^{1}H-NMR, mass spectrometry and confirmed by X-ray analysis. The physicochemical data lead to the identification of 3,7-dimethyl-1H, 9H,10H-di-pyrano[4,3-b:3′,4′-e]pyran-1,9-dione.

INTRODUCTION

Improvements in cosmetic quality control have resulted in rapid and reliable procedures for the evaluation of preservative agents in raw materials and in finished products (1–3).

It is well known that a mixture of preservatives generally has a wider profile of activity against microorganisms than that of each constituent of the mixture. Furthermore, the resulting toxicity may be lower, due to the smaller amount of each constituent used, in comparison with its use as a single component.

In order to test the stability of such mixtures, we recently reported a comparative study of the behavior of dehydroacetic acid sodium salt (I) (DHA.Na) (Prevan®), when used with imidazolidinylurea (Germall 115®), bromonitropropandiol (Bronopol®), and isothiazolinone (Kathon CG®) derivatives, in preservative standard mixtures (4) and in cosmetic products (5). On that occasion we emphasized the presence of an interaction product between DHA.Na and formaldehyde released from Bronopol® and Germall 115®.

In the present paper we describe the chemical interaction between DHA.Na and free or released formaldehyde. The structural characterization of the compound which originates from this reaction has been obtained by ^{1}H-NMR, mass spectrometry, elemental analysis and confirmed by X-rays (Bandoli *et al.*, in preparation). The presence of the interaction product has been proved by HPLC methods also in cosmetic emulsions.
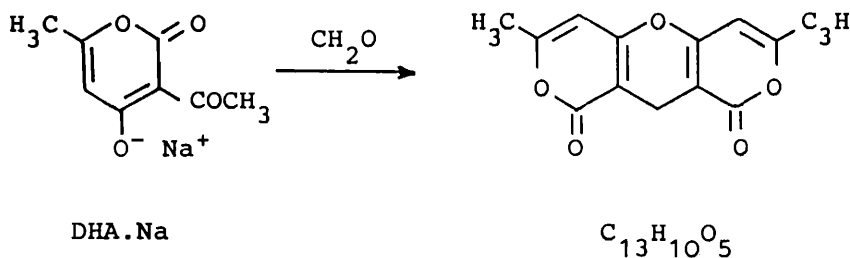
Scheme I: The reaction of dehydroacetic acid with formaldehyde.

EXPERIMENTAL

MATERIALS

Bronopol® (2-bromo-2-nitropropane-1,3-diol) (Formenti, Milano, Italia); formaldehyde 40% RPE (Carlo Erba, Milano); Germall 115® (N,N′-methylenebis[N′(1-hydroxy-methyl)2,5-dioxo-4-imidazolidinylurea)], (Medolla, Milano); Prevan® (3-acetyl-6-methyl-2H-pyran-2,4(3H)-dione sodium salt) (Formenti, Milano).

STANDARD AQUEOUS SOLUTIONS

DHA.Na 1.5 mg/ml; formaldehyde from 0.1 to 2.0 mg/ml; DHA.Na-Bronopol® 1.5 and 0.1 mg/ml, respectively; DHA.Na-Germall 115® 1.5 and 2.5 mg/ml; DHA.Na-formaldehyde 1.5 and 0.2 mg/ml.

SAMPLES

About 1 g, accurately weighted, of each cosmetic emulsion was diluted to 10 ml with a tetrahydrofuran/water solvent mixture (THF/H$_2$O 9/1).

METHODS

HPLC. All experiments were carried out in reversed phase mode using a Perkin Elmer S-4 liquid chromatograph equipped with an LC-85 UV detector and a Sigma 15 data station.

Chromatographic parameters:
Method 1a: Li-NH$_2$ (10 μm) Merck column, 1.0 ml/min flow rate, 216 nm UV detection, and acetonitrile/phosphate buffer 0.01 M, pH 4.7 (55/45%) mobile phase, 6 μl injection volume.
Method 1b: Li-NH$_2$ (10 μm) Merck column, 1.0 ml/min flow rate, 216 nm UV detection, and acetonitrile/phosphate buffer 0.01 M, pH 4.7/phosphoric acid 0.01 M (55/42/3%) mobile phase, 6 μl injection volume.
Method 2: RP-8 (10 μm) Merck column, 1.0 ml/min flow rate, 300 nm UV detection, and acetonitrile/phosphate buffer 0.01 M, pH 4.7 (50/50%) mobile phase, 6 μl injection volume.

¹H-NMR. ¹H-NMR spectra were recorded on a Varian FT-80A spectrometer with the

following procedure: frequency 80 MHz; mode FT; lock internal from $CDCl_3$; temperature 31°C; solvent $CDCl_3$ with 1% v/v TMS as internal standard; concentration about 0.1 M; tube size 5 mm O.D.; pulse width 58 µs: acquisition time 1 sec; spectral width 1500 Hz; no. of transients 100; no. of data points 4K (16K with zero filling before FT). Coupling constants are given in Hz; the relative peak areas, the decoupling experiments, and calculations of the chemical shifts using additivity rules were in agreement with all assignments.

Mass Spectrometry. All measurements were performed on a VG ZAB 2F mass spectrometer operating in Electron Impact (EI) conditions (70 eV, 200 µA). Samples were introduced via direct inlet system with an ion source temperature of 200°C. Metastable transitions were obtained by B/E linked scans (6). Exact mass measurements were performed by the peak matching technique at 10,000 resolution (10% valley definition).

RESULTS AND DISCUSSION

Our observation that some formulations could not prevent the development of molds over a long period of time suggested the possible degradation of the preservative system. Hence we undertook a study of the kinetics of disappearance of DHA.Na vs time, either alone or in a mixture with formaldehyde releasers (Bronopol® or Germall 115®) via HPLC.

The results, reported in Figure 1a, showed that there is a strong decrease in the concentration of DHA.Na in the presence of Bronopol® or Germall 115® in comparison with control samples of DHA.Na alone. It was postulated that this decrease was due to an interaction between DHA.Na and the released formaldehyde, giving rise to the formation of a new product. In fact, the HPLC runs of binary mixtures showed a third product, whose concentration is time-dependent (see Figure 1b).

To confirm the involvement of formaldehyde in the reaction, we carried out a series of tests on aqueous solutions of DHA.Na and formaldehyde in different molar ratios. The results, reported in Figure 2, fully support our hypothesis. The observed peak due to the interaction product was confirmed by various HPLC methods to be a unique product (4) whose structure must be determined as a first necessary step for further toxicological studies. The unknown product (compound 1) is obtained as a precipitate in significant amount (1.4 g) with a 25% yield by long-time (4 weeks) reaction of aqueous equimolecular (0.08 M) solutions of DHA.Na and formaldehyde at room temperature. The product, recrystallized from chloroform, does not melt up to 340°C. The elemental analysis gave: C, 63.44%; H, 3.99%, which is in accordance with a molecular formula: $C_{13}H_{10}O_5$ (Calculated: C, 63.41%; H, 4.09%). The corresponding molecular weight was confirmed by mass spectrometry. In fact, the 70 eV electron impact mass spectrum (Figure 3) shows the most abundant peak at m/z 246, in agreement with a molecular ion $[C_{13}H_{10}O_5]^{+\circ}$. Furthermore, accurate mass measurements gave a value of 246.0534 (± 0.002), in agreement with the molecular formula $C_{13}H_{10}O_5$ (246.0525).

The ^{1}H-NMR spectrum (Figure 4) shows a quartet at δ 5.88 with a coupling constant of 0.8 Hz, a broad singlet at δ 3.29 with the same unit integral, and a doublet at δ 2.26
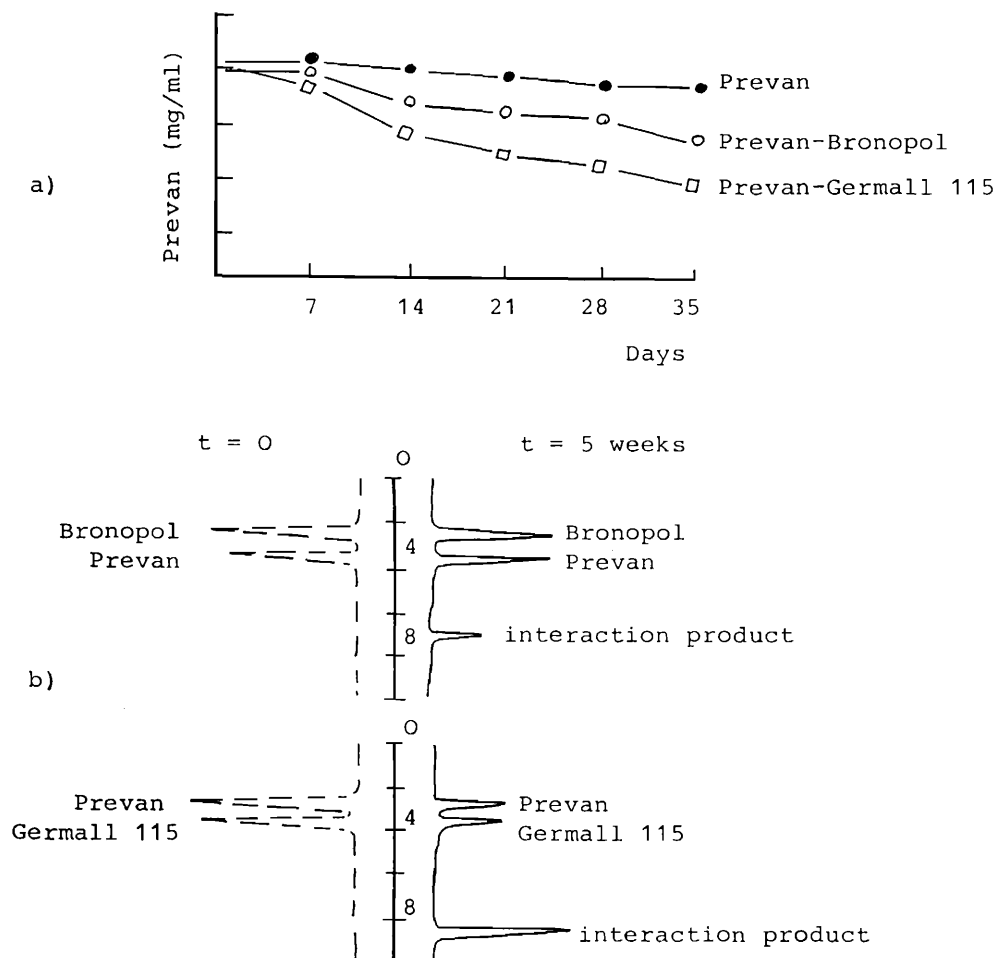
Figure 1. a) Trend of DHA.Na concentrations vs. time. b) HPLC runs of binary mixtures DHA.Na-Bronopol® (see method 1a) and DHA.Na-Germall 115® (see method 1b).

with an integral three times each of the two mentioned above. The appearance of the spectrum is very dissimilar to that of DHA (Figure 5): a one-proton singlet at δ 16.60, a one-proton quartet at δ 5.93 with coupling constant of 0.7 Hz, a singlet at δ 2.67, and a doublet at δ 2.29, both with a three-proton integral. Noticeable is the disappearance in the spectrum of compound 1 of the acetyl group at δ 2.67, and the maintenance of the methyl group and that of the corresponding coupled (J = 0.7 Hz) proton at δ 2.26 and δ 5.88, respectively. The other noticeable feature is the great variation of chemical shift from δ 16.60 to δ 3.29 for the remaining peak. In the first case it can be due to a very deshielded proton, attributable to the acidic hydrogen atom in the 3-position, while in the second it could originate from a slightly deshielded aliphatic proton(s). According to the molecular weight, the molecule should have a symmetry plane, and therefore the latter peak must be attributable to a methylene moiety. From
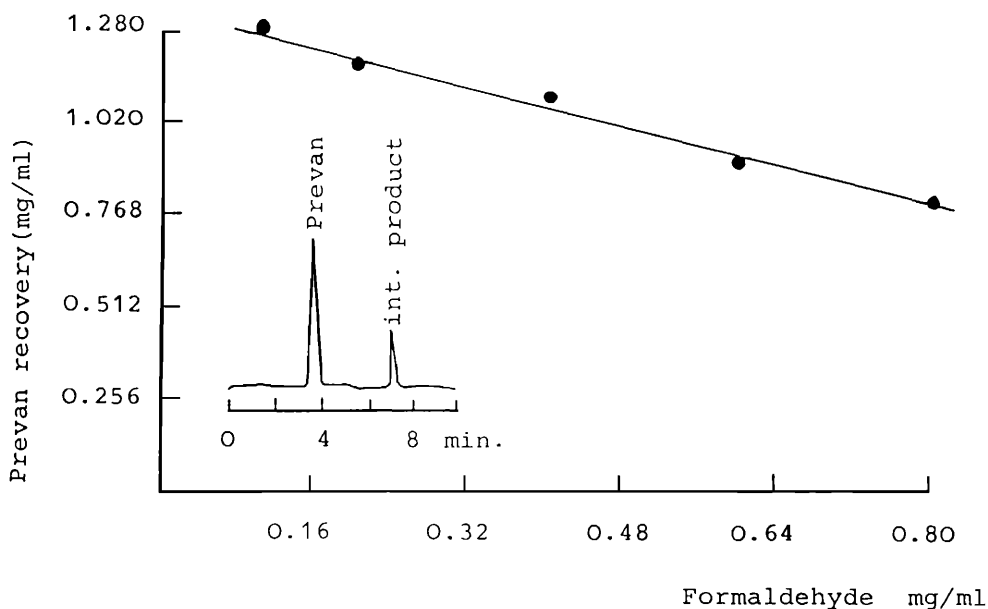
Figure 2. Chromatographic pattern of a DHA.Na/formaldehyde water solution five weeks after storage and DHA.Na behavior in dependence on formaldehyde concentration.

the above-reported data and from comparison with simple calculations of chemical shift, based on the usual additivity rules, we suggest the structure 3,7-dimethyl-1H,9H,10H-dipyrano[4,3-b:3′,4′-e]pyran-1,9-dione (7).

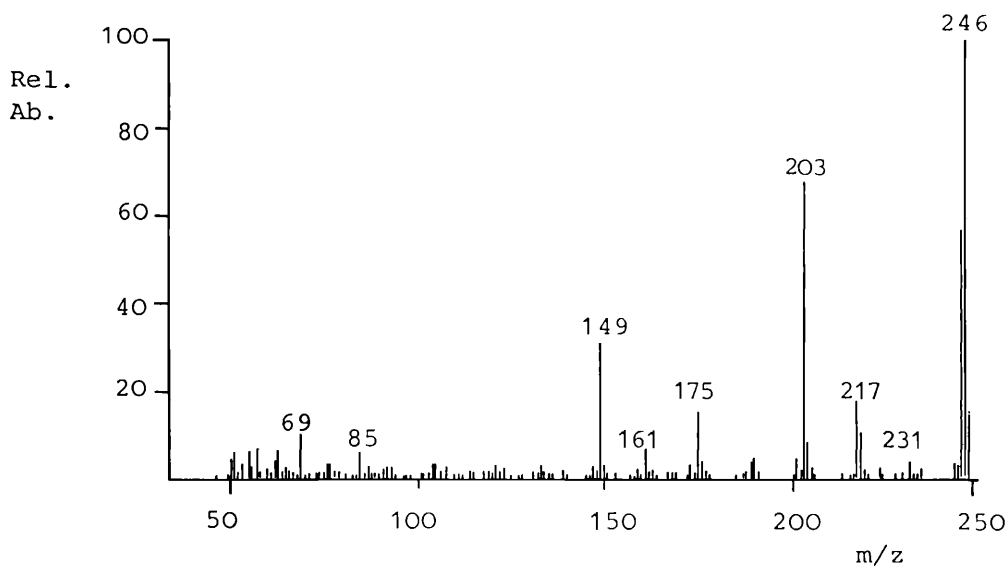The 70 eV EI mass spectrum of compound 1 is shown in Figure 3, while the related

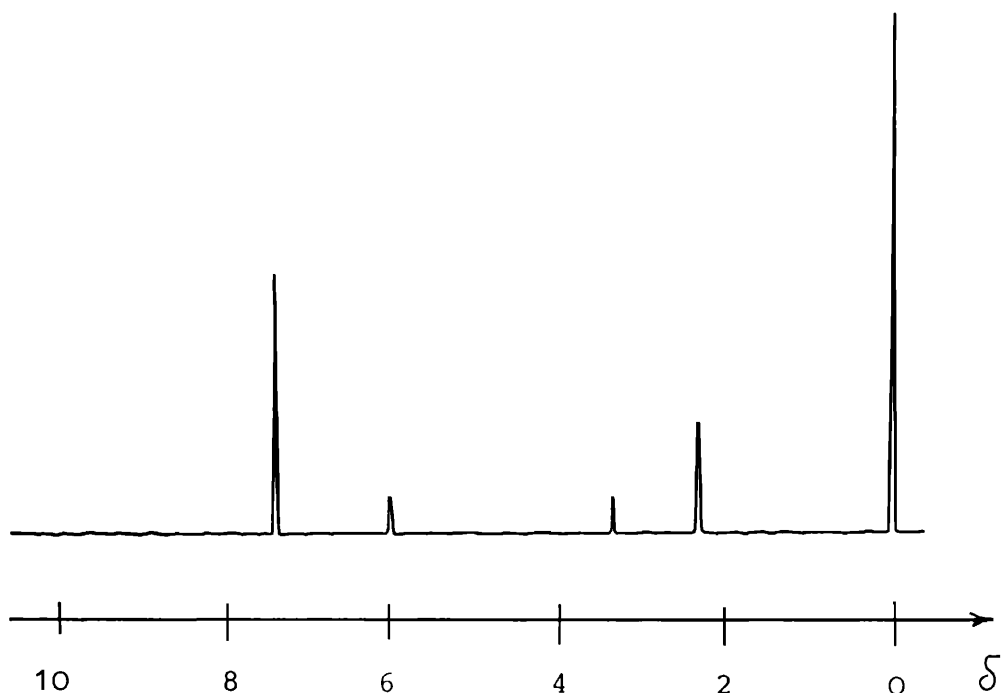**Figure 3.** 70 eV EI mass spectrum of compound 1.

Figure 4. ^{1}H-NMR spectrum of compound 1.

fragmentation pattern is reported in Scheme II. Also in this case a good relationship between the proposed structure and the EI-induced fragmentation pattern has been found.

Most of the total ion current is retained by the molecular ions at m/z 246, which give rise to the base peak of the mass spectrum. This behavior is in agreement with the aromatic-like proposed structure. The primary fragmentation pathway consists in methyl loss, leading to $[C_{12}H_7O_5]^+$ ions at m/z 231 and to CO and CHO· losses. These last two fragmentation pathways result quite typically in EI mass spectrometry of quinones and pyranones (8). For the ions at m/z 218, the formation of a substituted furan
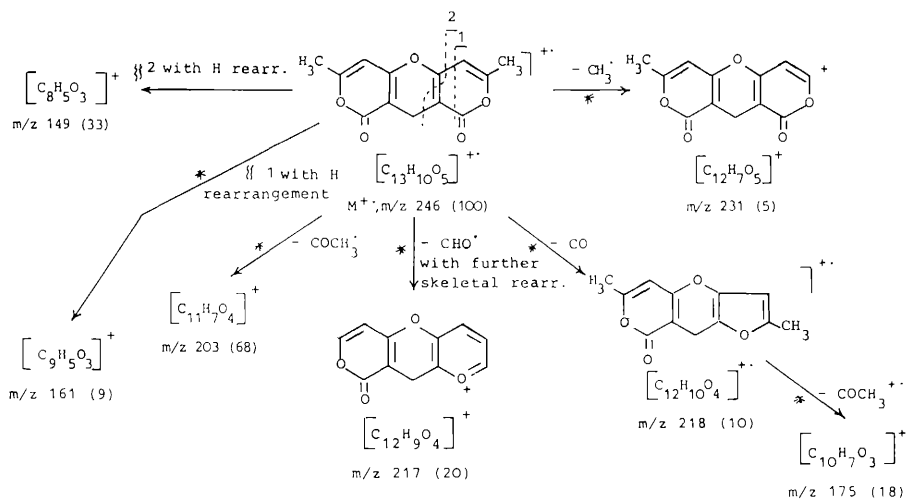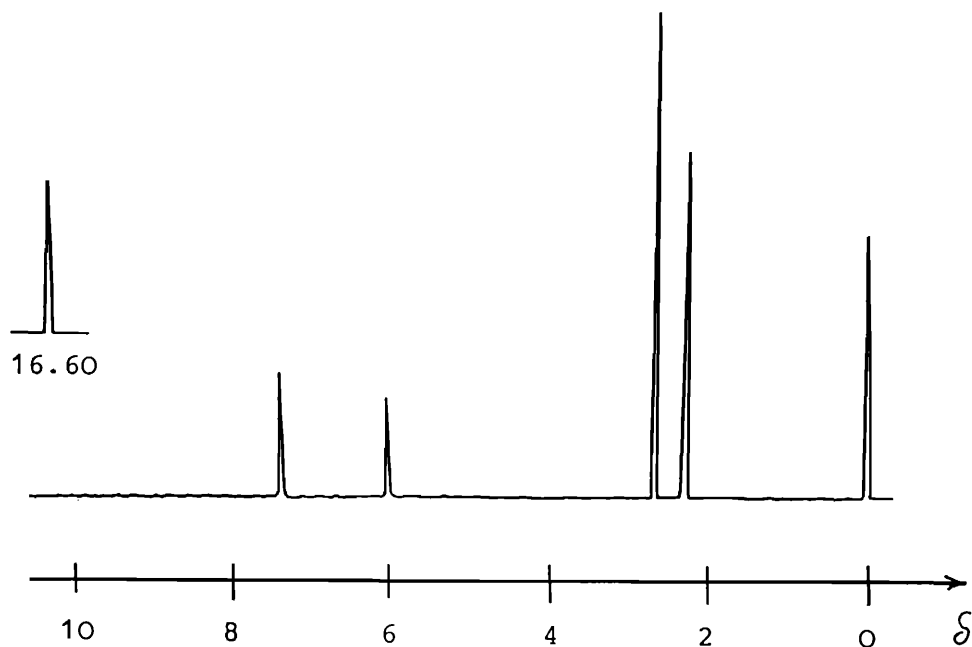
Figure 5. ^{1}H-NMR spectrum of DHA.

ring can be invoked, due to the higher stability of such a polycyclic structure. Analogously, for the ions at m/z 217 the formation of a pyranyl cation can be proposed. Finally, the cleavages 1 and 2 reported in Scheme II, of the polycyclic skeleton lead to ionic species $[C_9H_5O_3]^+$ (m/z 161) and $[C_8H_5O_3]^+$ (m/z 149) respectively, while the primary CH_3CO loss leads to $[C_{11}H_7O_4]^+$ ions.

Having determined the structure of the reaction product of DHA.Na and formaldehyde in aqueous solutions, we thought it of interest to test its presence in cosmetic formulations containing DHA.Na and Bronopol® or Germall 115®.

HPLC runs of a standard solution of compound 1 and of cosmetic emulsions (3 months stored at room temperature) preserved with Prevan-Bronopol® and Prevan-Germall 115® are reported in Figure 6.

The cosmetic samples were diluted 1:10 with THF/H_2O (9/1) before HPLC analysis (9). In their chromatographic patterns there clearly appears a well-marked peak presenting the same $k' = 1.76$ of compound 1. In order to confirm its structure we collected the corresponding peak fractions that had undergone mass spectrometry analysis. The spectra, in accordance with the standard, gave unambiguous evidence of the reaction between DHA.Na and CH_2O released by preservatives. Other ingredients of the cosmetic formulation did not affect the formation of 3,7-dimethyl-1H,9H,10H-dipyrano[4,3-b:3′,4′-e]pyran-1,9-dione.

CONCLUSIONS

An interaction product between DHA.Na and formaldehyde released from other preservatives has been demonstrated by HPLC methods using aqueous solutions or cosmetic formulations. The structure of this unexpected product appears to be: 3,7-dimethyl-1H,9H,10H-dipyrano[4,3-b:3′,4′-e]pyran-1,9-dione, by elemental analysis, ^{1}H-NMR, mass spectrometry and X-ray analysis.

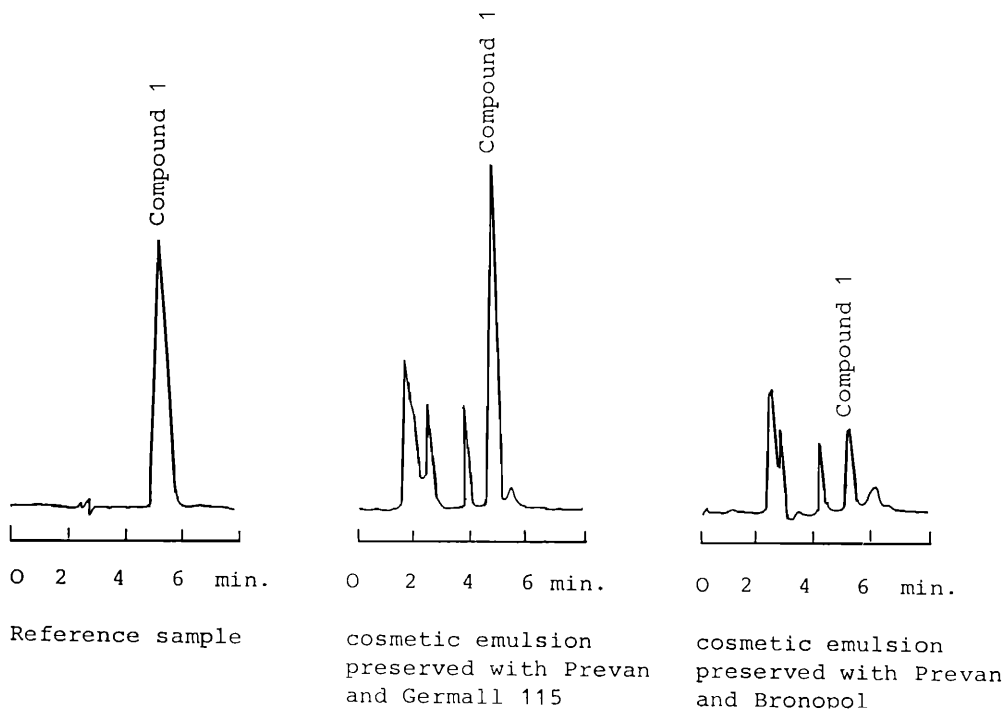As this structure has been first postulated (unknown) in the past, the degradation product exhibits a

Figure 6. HPLC runs of cosmetic emulsions preserved with DHA.Na-Bronopol® and DHA.Na-Germall 115® after dilution with THF/H₂O (see method 2).

molecular weight higher than DHA, due to the reaction of two molecules of DHA with formaldehyde.

ACKNOWLEDGMENT

REFERENCES

(1) A. Bettero, A. Semenzato, G. Aversa, and C. A. Benassi, Imidazolidinilurea e formaldeide nei prodotti cosmetici, *Chimica Oggi,* 11, 29–32 (1985).
(2) A. Bettero, F. Galiano, S. Daolio, and C. A. Benassi, The characterization of isothiazolinone preservatives in cosmetics, *J. Pharm. Biomed. Anal.,* 3, 581–587 (1985).
(3) A. Bettero, A. Semenzato, and C. A. Benassi, Preservatives in cosmetics. Developments on direct characterization, *Proc. 14th IFSCC,* Barcelona, 16–19 September 1986, Vol. I, 187–194.
(4) C. A. Benassi, A. Semenzato, M. Lucchiari, and A. Bettero, Dehydroacetic acid sodium salt stability in cosmetic preservative mixtures. *Int. J. Cosmet. Sci.* 1988 (in press).
(5) C. A. Benassi, A. Bettero, A. Semenzato, and R. Cerini, Correlazione tra analisi chimica e previsione di stabilità microbiologica di un prodotto cosmetico. *Proc. VII Congresso Naz. Div. Chim. Farm. Soc. Chim. It.,* Pisa, 22–26 Settembre 1987.
(6) A. P. Bruins, K. R. Jenning, and S. Evans, The observation of metastable transitions in a double-focussing mass spectrometer using a linked scan of the electric sector and magnetic sector fields, *Int. J. Mass Spectrom. Ion Phys.,* 26, 395–404 (1978).
(7) M. Moreno-Mañas, J. Ribas, and A. Virgili: The 3,6,9-trioxanthracene and 3,6-dioxa-9-thianthracene ring systems by cyclization of 1,1-bis[4-hydroxy-6-methyl-2-oxo-2H-pyran-3yl] alkanes, *Synthesis,* 3, 699–701 (1985).
(8) Q. N. Porter, *Mass Spectrometry of Heterocyclic Compounds* (Wiley, New York, 1985), pp. 198–211.
(9) A. Bettero, B. Casetta, F. Galiano, E. Ragazzi, and C. A. Benassi, Rheological and spectroscopic behaviour of cosmetic products: first application in sulphur analysis. *Fresenius Z. Anal. Chem.,* 318,