enzyme activity in pituitary gland at 5 weeks of age was the highest among the three ages tested. However, in the other brain regions, the activity of this enzyme was highest at 3 weeks old. These results confirm previous data concerning the developmental changes in the activity of rat whole brain[2]. These changes in developing rat whole brain are unique for the dipeptidyl aminopeptidase represented here, while other dipeptidyl aminipeptidases (dipeptidyl peptidase I and III) showed little changes in developing rat whole brain[1]. In various rat brain regions, the activity in the pons-medulla was slightly higher at 3 weeks old, but was similar to other brain regions at 5 and 20 weeks (table 1).

Changes in the enzymatic activity in developing rat brain regions were similar to those in the whole brain. These results indicate that dipeptidyl aminopeptidase has a broad distribution, but do not show whether the enzyme is located in neuronal or in glial cells.

Table 2. Subcellular distribution of dipeptidyl aminopeptidase activity in rat cerebrum, hypothalamus, midbrain and pons medulla

| Subcellular fraction | | Percent of total activity | | | |
|---|---|---|---|---|---|
| | | 1 week | 3 weeks | 5 weeks | 20 weeks |
| Cerebrum | $P_1$ | (17.7) | 20.0 | 9.0 | 23.1 |
| | $P_2$ | (54.0) | 36.8 | 44.5 | 33.9 |
| | $P_3$ | ( 6.6) | 6.2 | 6.1 | 6.0 |
| | Sup | (21.7) | 15.8 | 8.1 | 11.6 |
| Hypothalamus | $P_1$ | (22.3) | 18.3 | 12.9 | 17.8 |
| | $P_2$ | (61.5) | 30.1 | 23.0 | 33.7 |
| | $P_3$ | ( 3.5) | 0.7 | 1.8 | 1.1 |
| | Sup | (12.7) | 22.7 | 21.5 | 18.8 |
| Midbrain | $P_1$ | (18.3) | 21.3 | 9.2 | 26.8 |
| | $P_2$ | (48.9) | 29.3 | 42.2 | 30.7 |
| | $P_3$ | ( 8.8) | 6.8 | 9.7 | 6.1 |
| | Sup | (24.0) | 11.9 | 10.7 | 15.6 |
| Pons medulla | $P_1$ | (24.0) | 18.2 | 9.0 | 24.3 |
| | $P_2$ | (51.0) | 26.7 | 39.1 | 35.0 |
| | $P_3$ | ( 7.2) | 3.9 | 8.8 | 4.2 |
| | Sup | (17.8) | 11.5 | 11.3 | 10.6 |

Values were calculated from total activity in a homogenate fraction, and represent means from 3 experiments.
The values for 1-week-old rat brain, given in parentheses, were estimated as a percentage of the gross activity in each fraction.
Symbols used in this table represent subcellular fractions; $P_1$, $P_2$ and $P_3$, pellets at 700 × g, 10,000 × g, and 100,000 × g, respectively; Sup, supernatant at 100,000 × g.

Subcellular distribution of dipeptidyl aminopeptidase in 4 brain regions is shown in table 2. The activity in the $P_1$ (nuclear) fraction is slightly high, but this might result from contamination with intact cells in addition to nulei and cell debris. The activity in the $P_2$ (mitochondrial) fraction is about one half of the total activity in each of the brain regions studied, and the activity in the $P_3$ (microsomal) fraction is the lowest in the 4 fractions, during maturation of rat brain. The $P_2$ fraction consists of synaptic vesicles and myelin, in addition to membrane debris, mitochondria and lysosomes, so it is probable that dipeptidyl aminopeptidase in the brain is present in the nerve endings. In this study, rather high enzyme activity is observed in the supernatant fraction. It may be derived from the activity in particles of the $P_2$ fraction because of the similarity in optimum pH (data not shown). Since rat brain dipeptidyl aminopeptidase attacks peptide bonds between *N*-terminal X-Pro dipeptides (X: amino acid residues) and amino acid or arylamide, a neuropeptide containing the X-Pro sequence at the *N*-terminal position may be susceptible to hydrolysis by this enzyme. These results indicate the possibility that the processing of biologically active peptides occurs in the nervous system.

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## A comparison between skin-photosensitizing (334 nm) activities of 8-methoxypsoralen and angelicin

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*Summary.* 8-Methoxypsoralen and angelicin, locally applied to the skin of the rabbit, photosensitized erythema under UV irradiation to an approximately equal extent. Incident UV irradiation was restricted mainly to 334 nm. This indicates that furocoumarin-DNA cross-links are not important in erythema induction.

Furocoumarins (psoralens) photosensitize erythema of the mammalian skin under near-UV irradiation (UVA, 315–400 nm)[2]. The linear furocoumarins such as 8-methoxypsoralen (8-MOP) are known to photosensitize erythema under monochromatic light (366 nm) much better than those with the angular structure, for instance, angelicin[2,3]. This difference was thought to be due to the ability of 8-MOP to form cross-links with DNA under UVA-irradiation, whereas angelicin has no such ability[4,5]. It should, however, be noted that molar absorbtion coefficients ($\varepsilon_{366}$) for 8-MOP and angelicin at 366 nm are 1000 and 60 $1 \times mol^{-1} \times cm^{-1}$, respectively. This means that the lower photosensitizing activity of angelicin, compared to that of 8-MOP, may be due to its lower absorbance at 366 nm, and not due to the inability of angelicin to form cross-links.

The purpose of the present paper is to compare the photosensitizing activities of 8-MOP and angelicin irradiated at 334 nm, where the $\varepsilon_{334}$ values of both are approximately equal at 4000 $1 \times mol^{-1} \times cm^{-1}$.

8-MOP and angelicin were kindly supplied by Professor G. Rodighiero (Padova, Italy).

Albino rabbit skin was used. The rabbit's back was shaved

with a safety-razor 24 h before the treatment. 0.1 ml of an ethanol solution of 8-MOP or of angelicin, at the concentration of $5 \times 10^{-3}$ mol $\times$ l$^{-1}$, was applied to an area of skin within a polyethylene ring of 1.7 cm diameter. The solute was evaporated with a stream of air for 1-2 min[6]. Ethanol was applied to the control skin areas. The surface concentration of the substances applied was $2.2 \times 10^{-7}$ mol $\times$ cm$^{-2}$. The skin was UV-irradiated 40 min after application of the photosensitizers. A SVD-120A high pressure mercury lamp was used, with a glass filter, and a water solution of $NiSO_4$ and $CoSO_4$ as another filter. The filters restricted the incident irradiation mainly to 334 nm. Quantum fluence rates of the incident radiation were distributed (in percentages of the total fluence rate) as follows: 10%, 63% and 27%, for the wavelengths 313, 334, and 366 nm, respectively, as estimated with a rhodamine quantum counter[7]. The total fluence rate of UV radiation was $1.82 \times 10^{18}$ quanta $\times$ sec$^{-1}$ m$^{-2}$ (corresponding to 1.03 J $\times$ sec$^{-1}$ m$^{-2}$ as determined by ferrioxalate actinometry[7]. The criterion of the erythematous response was the minimal erythema dose (MED), reddening observed in 72 h after the irradiation. Five areas treated with 8-MOP or angelicin were irradiated in order to establish MED. The fluence was enhanced by 30% for every other area of skin.

Results obtained with 9 rabbits are summarized in the table. The absolute skin photosensitivity of individual animals is known to vary significantly. That is why we introduced one more parameter - the ratio of the MED of skin treated with 8-MOP ($MED_{8\text{-}MOP}$) to the MED of skin treated with angelicin ($MED_{ang}$), for each animal. As is seen from the table, the erythema-inducing property of angelicin under irradiation at 334 nm is comparable to that of 8-MOP. A 60-min irradiation (3700 J $\times$ m$^{-2}$) of the skin treated with ethanol alone caused no reddening. The known values of photosensitizing activities, namely 37 and 12 (relative units) for 8-MOP and angelicin, respectively, under irradiation at 366 nm (2) may be accounted for by the considerable difference in the $\varepsilon_{366}$ values of these 2 substances.

In our experiments 8-MOP and angelicin were shown to exhibit approximately equal photosensitizing activities under irradiation at 334 nm. Consequently, cross-linking with DNA is not important in furocoumarin + UVA-induced erythema.

A comparison between skin photosensitizing activities of 8-MOP and angelicin. Incident irradiation is restricted mainly to 334 nm. $MED_{8\text{-}MOP}$, and $MED_{ang}$ are minimal erythema doses for 8-MOP + UVA and angelicin + UVA, respectively

| Animal No. | $MED_{8\text{-}MOP}$ (J m$^{-2}$) | $MED_{ang}$ (J m$^{-2}$) | $\frac{MED_{8\text{-}MOP}}{MED_{ang}}$ |
|---|---|---|---|
| 1 | 1483 | 803 | 1.85 |
| 2 | 1483 | 1483 | 1.00 |
| 3 | 536 | 803 | 0.67 |
| 4 | 803 | 536 | 1.50 |
| 5 | 803 | 357 | 2.25 |
| 6 | 1112 | 1483 | 0.75 |
| 7 | 674 | 781 | 0.88 |
| 8* | 1112 | > 1483 | |
| 9* | 556 | < 742 | |
| Mean ± SD | 987 ± 144 | 892 ± 165 | 1.27 ± 0.23 |

*These values were not taken into account in the ratio $\frac{MED_{8\text{-}MOP}}{MED_{ang}}$.

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## Effectiveness of phosphocitrate and N-sulpho-2-amino tricarballylate, a new analogue of phosphocitrate, in blocking hydroxyapatite induced crystal growth and calcium accumulation by matrix vesicles

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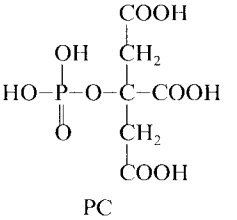
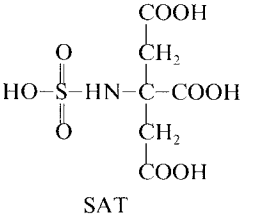
*Summary.* Phosphocitrate and its analogue N-sulpho-2-amino tricarballylate were compared with ethane-1-hydroxy-1,1-diphosphonate for inhibition of calcium phosphate crystallization in hydroxyapatite induced crystal growth and $^{45}Ca$ uptake by matrix vesicles. Phosphocitrate (1 μM) was the most potent inhibitor followed by ethane-1-hydroxy-1,1-diphosphonate and N-sulpho-2-amino tricarballylate, the latter requiring a high concentration (100 μM) to be equally effective as an inhibitor.

Biological mineralization is a complex phenomenon whereby calcium phosphate salts are transformed to hydroxyapatite (HA), a process that may be influenced by natural inhibitors in the microenvironment. Phosphocitrate (PC) is one of the most potent natural inhibitors but as yet its potential in vivo has not been thoroughly investigated. However, because PC may be prone to enzyme hydrolysis[2], a search for new stable analogues to PC was initiated. One such analogue N-sulpho-2-amino tricarballylate (SAT) has now been synthesized which is a little less active than PC as an anticalcifying agent[3]. Nevertheless, its stability in vivo could enhance its usefulness.



PC



SAT