## Sur un nouveau type de dégradation enzymatique de l'arginine: l'oxydation en guanidobutyramide

Des préparations de *Streptomyces griseus* (Waksman) (homogénat de mycélium préalablement lavé à l'eau distillée), incubées en présence d'arginine à pH = 7.4 sous barbotage d'un mélange de 95% $O_2$ + 5% $CO_2$, oxydent cet acide aminé: l'analyse chromatographique[1] du milieu réactionnel montre la présence d'un dérivé guanidylé de $R_F$ différent de celui des corps guanidiques antérieurement décrits (agmatine, acide arginique, glycocyamine, acides guanidopropionique, guanidobutyrique, guanidocétovalérianique, entre autres).

Le nouveau corps a été cristallisé à l'état de sulfate sous forme d'aiguilles, P.F.: 145–146°, et analysé. Il donne avec l'hydroxylamine et le perchlorure de fer la réaction des acides hydroxamiques (réaction de BERGMANN). Hydrolysé en présence d'acide chlorhydrique, il donne naissance à de l'ammoniac et à l'acide guanidobutyrique et sa composition élémentaire correspond à celle de l'amide de l'acide guanidobutyrique (sulfate).

Ce dernier, préparé par synthèse en traitant le guanidobutyrate d'éthyle par de l'ammoniaque, présente les mêmes caractéristiques chromatographiques, le même point de fusion (P.F.: 145°–146°) et la même composition élémentaire que le corps naturel isolé.

| | *Produit naturel* | *Produit synthétisé* | *Calculé pour le sulfate d'amide guanidobutyrique* |
|---|---|---|---|
| C | 25.1 | 25.2 | 24.8 |
| H | 6.0 | 6.1 | 5.8 |
| O | 32.0 | — | 33.1 |
| N | 22.4 | 22.6 | 23.1 |

Ce dérivé se forme directement par oxydation de l'arginine, sans libération d'ammoniac dosable (méthode de CRISMER). Sous azote, même en présence d'acide adénosinetriphosphorique, on n'observe la production de ce corps ni à partir de l'arginine, ni à partir de l'acide guanidobutyrique en présence d'ammoniac. Les inhibiteurs habituels des réactions d'amidination (fluorure, hydroxylamine) ou de la L-aminoacideoxydase (cyanure de potassium, azide de sodium) n'ont aucun effet sur sa formation.

L'acide guanidobutyrique, qui prend naissance à côté de l'amide, dans certaines préparations, semble provenir de l'hydrolyse enzymatique du dernier produit et non de l'oxydation spontanée de l'acide δ-guanido-α-cétovalérianique formé par action de la L-aminoacideoxydase sur l'arginine. En effet nous n'avons pas noté la présence de ce dernier dans les milieux réactionnels, alors que l'on observe facilement lorsque la L-aminoacideoxydase est présente[2,3].

La formation de la guanidobutyramide est ainsi attribuée à un nouveau type d'enzyme, susceptible d'oxyder l'arginine au niveau du carbone C 2, en entraînant une décarboxylation de l'acide aminé tout en en laissant le groupe aminé intact.

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## "Sun-screening" effect of urocanic acid

Considerable amounts of urocanic acid (UA)—imidazoleacrylic acid—have been demonstrated[1], on the basis of paper chromatographic and spectrographic evidence, to occur in human sweat. This substance strongly absorbs in the ultra-violet (U.V.). It would therefore seem probable that it might have some role in the protection of the skin against U.V. radiation. According to the findings of HAUSSER AND VAHLE[2], confirmed by others, a maximum of the erythemogenic activity is situated between 250 and 300 mμ. U.V. light of longer wave lengths (300–430 mμ), on the other hand, has a beneficial effect and supports the formation of melanin pigment which represents the natural protection of the skin.

The purpose of this paper is to find out whether UA of sweat exerts any significant "sun-screening" effect in protecting the skin against the erythema-producing radiation but transmitting the pigment-producing wave lengths.

According to DORNO[3], in Davos (1600 m above the sea level) the lowest limit of the solar spectrum is 307 mμ in winter and 290 mμ in summer (midday) (Fig. 1 A). Curve B (Fig. 1) shows

the dependence of the erythemogenic activity on the wave length, according to HAUSSER AND VAHLE[2]. Only the second maximum (290-310 mμ) is important for terrestrial conditions. Curve C (Fig. 1) shows the pigmentation-producing effect of the solar radiation (300-430 mμ) with a maximum at 320-350 mμ. Curve D (Fig. 1) illustrates the permeability of the epidermis[2,4] in terms of transmission (%) and shows that the epidermis offers only partial protection in the erythemogenic spectral range.

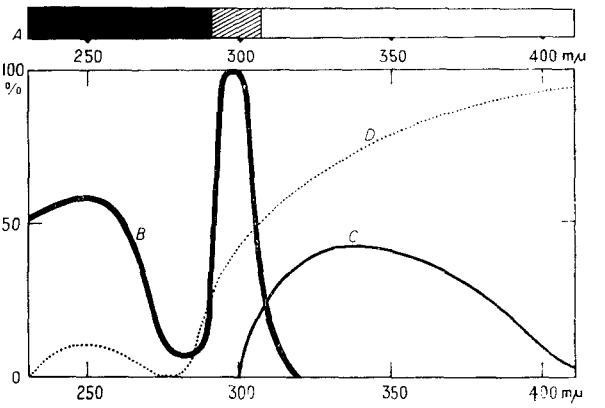


Fig. 1. A, lowest limits of the solar spectrum at 1600 m according to DORNO[3] (black area represents the wave lengths which do not penetrate to the surface even in summer, striated area shows the borderline range); curve B, erythemogenic activity according to HAUSSER AND VAHLE[2] expressed as percentage of activity at 295 mμ; curve C, tanning activity according to HAUSSER AND VAHLE[2]; curve D, permeability of the epidermis in terms of percent transmission[2,4].

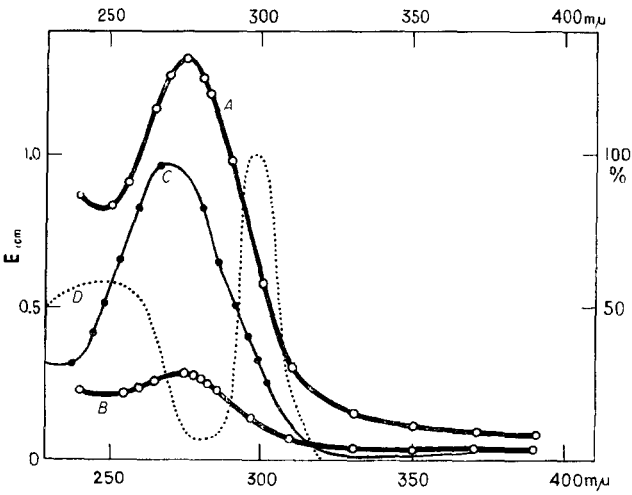


Fig. 2. Curve A, optical density of a five times diluted sample of sweat (final concentration 8 μg UA per ml, according to paper chromatography), 1 cm light path, pH 7; curve B, same sample as A after electrolytically desalting the sweat; curve C, optical density of a solution of 8 μg anhydrous UA made nearly neutral (pH 7.1) with a small amount of 0.1 N-NaOH, 1 cm light path; curve D, erythemogenic activity[2] (identical with curve B, Fig. 1) with ordinate on the right hand side of the figure.

UA concentration in sweat of 15 subjects was determined using quantitative paper chromatography[5], and values in the range of 40–160 μg/ml (mean value 170 ± 37 μg/ml) were found, the accuracy of the method being ± 20%.

Curve A in Fig. 2 shows the absorption of U.V. radiation by five times diluted human sweat; the original concentration of UA in the sample tested was 40 μg/ml. Absorption maximum near 275 mμ can be observed. Curve B (Fig. 2) shows the decrease of absorption after electrolytically desalting the sweat, due to the reduction of UA to imidazolepropionic acid[1] which does not contain the acrylic double bond and does not absorb in this range.

In order to determine to what degree UA is responsible for this absorption of sweat, the extinction curve for the water solution of UA, with approximately the same concentration and the same pH as the sample of sweat, was calculated. As there are considerable discrepancies in the values found by various workers for the specific extinction of UA[6*] the spectrum measured by KAKÁČ using the Zeiss apparatus with rotating sector was taken as a basis for the graph. As may be seen in Fig. 2, the shape of the spectrum (curve C) coincides to a considerable degree, in height and position of the maximum with that of sweat. Most of the U.V. absorption of sweat can thus be attributed to the UA content (see also[10]). A better agreement could not be expected,

as the determination of the concentration of UA was only approximate and as the presence of other substances in sweat might influence the absorbing properties of UA.

It is interesting that in the range 300–310 mμ, which is of practical significance, the difference between the extinction of the non-desalted and desalted sweat noticeably surpasses the extinction of aqueous UA solution of similar concentration. This increase could be due to the presence of other substances in sweat.

When comparing the UA absorption curve (C, Fig. 2) with the curve of erythemogenic activity (D, Fig. 2), one sees that the maximum absorption of both the UA and the total sweat does not coincide with the maximum of biological activity; nevertheless the absorption of sweat in the range 295–305 mμ is not negligible.

From the data plotted in curve A (Fig. 2), we can calculate that already a 1 mm layer of sweat absorbs about 50% of the U.V. radiation at 300 mμ. It is evident that a thinner layer of a more condensed sweat would be sufficient for the same absorption. It is probable that such a thin layer of evaporated sweat, and perhaps an even more concentrated one, can be found on the skin of most people exposed to sun-rays and affords them a partial protection from sunburn. If they wash themselves or take a bath, this layer of UA is completely or partially washed off, and erythema may occur more easily. It is a well known fact that sunburn occurs more easily after swimming. It is even possible, although we have no evidence of it, that the absorption of the epidermis (see Fig. 1, curve D) is also partially due to the presence in the epidermal layer of UA which could be washed out with water.

The advantageous absorption properties of UA lead to the idea of protecting the skin by applying UA artificially[11], its absorption being equal to, or even stronger than many of the substances used in the commercial sun-screens. The natural occurrence of UA would suggest favourable pharmacological properties.

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* Molar extinction coefficient $\varepsilon = 1.84 \cdot 10^4$ published by MEHLER AND TABOR[7] or $1.88 \cdot 10^4$ found by MEHLER (private communication) is in line with that found by EDMUNDSON (private communication), namely $1.85 \cdot 10^4$ in a phosphate buffer at 277 mμ, pH 7.25. For aqueous solutions neutralized with NaOH $1.31 \cdot 10^4$ was found by HALL[8] (calculated for dihydrate), $1.35 \cdot 10^4$ by EDMUNDSON (private communication) and $1.67 \cdot 10^4$ by KAKÁČ (present paper). In comparing the various data, it must be kept in mind that the intensity of the double-bond U.V. absorption of UA may be affected by a number of other factors apart from the pH, such as the presence of extraneous substances or the action of light (C. PASINI, private communication; *cf.*[9]). Some of the discrepancies found in the literature are only apparent being due to ambiguous indication of the units.