plet, 2H: CH_2 en C-2), 2,13 ppm (singulet, 3H: CH_3–C(=O)–), 3,25 ppm (triplet, 2H: $-CH_2-O$-trityle), 3,30 ppm (singulet, 3H: OCH_3), 3,83ppm (quadruplet, 1H: $>CH-OCH_3$), 7,30 ppm (multiplet, 15H: H aromatiques).

Le composé **9** optiquement actif a été par ailleurs obtenu à partir de l'acide S (–)-malique au moyen de la séquence de réaction suivante. L'acide **10** a été transformé en *O*-méthyl malate de méthyle **11**[5]. La réduction de ce diester par $LiAlH_4$ dans l'éther anhydre fournit le méthoxy-2 butanediol-1,4 **12** $[\alpha]_D = -23,0°$ (c = 3,26, acétone) (lit.[5], $[\alpha]_D = -23,5°$). Le diol est traité par un 1,1 équivalent de chlorure de trityle dans la pyridine anhydre ce qui fournit un mélange des deux dérivés monotritylés **13a** et **13b** dans lequel le monotrityl **13a** est le produit prépondérant. Ces deux isomères ont été séparés par chromatographie sur gel de silice. Le composé **13a** non cristallisé $[\alpha]_D = +2,30°$ (c = 3,07, $CHCl_3$) a été oxydé par le réactif de JONES[4] en acide **14** $[\alpha]_D = -15,6°$ (c = 4,23, $CHCl_3$) qui se présente sous forme d'une huile incolore.

En traitant l'acide **14** par 3 équivalents de méthyllithium dans l'éther anhydre[6], on obtient un mélange de produits dont le composant prépondérant est la méthyl cétone **9b** $[\alpha]_D = -23,7°$ (c = 1,8, $CHCl_3$). Celle-ci présente le même Rf en chromatographie sur couche mince et le même spectre de RMN que le produit **9a** isolé à partir de l'acide bongkrékique. Le carbone C-17 de l'acide bongkrékique a par conséquent la configuration R, ce qui confirme l'assignation faite précédemment à partir de la courbe de dichroisme circulaire de l'acide bongkrékique[1,7].

Summary. The chiralities of the two asymetric centers of the toxic antibiotic bongkrekic acid have been determined: the C-6 center has the S configuration and the C-17 center the R configuration.

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[7] Tous les composés préparés au cours de ce travail ont présenté des spectres de RMN ($CDCl_3$, 60 MHz), de masse en accord avec leur structure.
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Integumental Distribution of Tyrosinase Activity in the Slow Loris, *Nycticebus coucang*, and the Presence of Integumental Tyrosinase Inhibitor(s)

In the study of melanin biosynthesis from an evolutionary viewpoint, relatively little is known of the primate pattern of integumental tyrosinase distribution. As the availability of the primates for such studies has been limited, the gift of a lower primate, the slow loris (*Nycticebus coucang*), provided a unique opportunity to extend our understanding of tyrosinase distribution. Thus, a study of the subcellular distribution of tyrosinase activity in various integumental regions differing in pigmentation as well as the eye was undertaken. Further, in the course of these studies it was discovered that preparations derived from certain skin areas also showed the presence of integumental tyrosinase inhibitor(s).

Methods and materials. Two female (451 and 549 g) and 1 male (454 g) loris (*Nycticebus coucang*) were sacrificed and the retinas and integumental areas (Table I) were removed, trimmed, weighed and frozen as previously described[1]. The usual enzyme preparation prodecure, radiometric assay, protein analysis and statistical procedures were utilized[1–3]. The activities of the enzyme preparations were dopa dependent and inhibited by sodium diethyldithiocarbamate (6 m*M*). The tyrosinase inhibitory activity was demonstrated by the incubation of mushroom tyrosinase with L-tyrosine-^{14}C in the presence and absence of skin enzyme preparations. A duplicate series of experiments using non-radioactive L-tyrosine to determine substrate concentration pre- and post-incubation with mushroom tyrosinase was also undertaken. The available L-tyrosine in the supernatant fraction post-trichloroacetic acid precipitation of protein was determined by the Folin-Ciocalteau procedure[4]. Wherever possible data are expressed in the form $\bar{X} \pm$ SEM.

Results and discussion. The tyrosinase activity varied anatomically in the skin (Table I). The highest enzymatic activities occurred in the genital skin areas and in the retina. The subcellular tyrosinase distribution differed in the retina and in the skin; the retina showed tyrosinase activity in both the particulate and soluble fractions whereas the skin tyrosinase activity was confined to the particulate fraction.

In the skin, the tyrosinase activities of the dorsal brown stripe, the left and right lateral trunk skin areas and the left and right ventral trunk skin areas were low. However, the enzymic activity was higher in the dorsal dark brown stripe than in the lateral or ventral skin. Interestingly, the tyrosinase activity of the ventral skin was higher than that of the lateral skin. Generally, the darker skin areas, such as the cranial skin, ears, black circumorbital ring and black area of male genital skin as well as the eye showed higher activity than the light brown colored skin area. In contrast, the light colored skin of the external female genitalia showed the highest tyrosinase activity and the relatively dark forehead skin area had very low activity.

In the homogenates, the specific activity of the enzyme was highest in the scalp, female external genitalia and the dorsum of the forepaw. In all integumental areas studied, the specific activity in the particulate fraction was higher than that in the homogenate. In some skin areas the particulate fraction tyrosinase activity exceeded that of the homogenate. The tyrosine carboxyl group incorporation into melanin was approximately 16% in the retina and ranged from 19 to 29% in the skin.

In the forepaw palm, interorbital streak and forehead, the enzymic activity of the particulate fraction was higher than that present in the homogenate from which

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Table I. Tyrosinase activity in the skin and retina of *Nycticebus coucang*

	TU[a]/mg tissue			Specific activity[b]			Tyrosine carboxyl group incorporation (%)[c]		
	H	P	S	H	P	S	H	P	S
Retina	298 ± 3	251 ± 4	56 ± 2	16.1 ± 1.3	16.4 ± 1.1	16.49 ± 0.93	12.8 ± 0.5	13.3 ± 0.4	13.1 ± 0.3
Dorsal dark brown stripe (trunk)	66 ± 2	66 ± 3	0	11.9 ± 0.5	26.6 ± 1.4	0	25.9 ± 1.4	25.9 ± 1.0	0
Left side (trunk)	24 ± 1	26 ± 2	0	3.6 ± 0.1	10.1 ± 0.6	0	20.0 ± 1.2	19.1 ± 1.0	0
Right side (trunk)	24 ± 1	24 ± 1	0	4.1 ± 0.0	11.6 ± 0.4	0	20.0 ± 1.0	20.0 ± 1.0	0
Ventral trunk, left side	48 ± 1	50 ± 2	0	6.4 ± 0.1	16.8 ± 0.6	0	17.9 ± 0.9	17.1 ± 0.9	0
Ventral trunk, right side	50 ± 2	51 ± 1	0	6.8 ± 0.1	17.9 ± 0.7	0	17.1 ± 0.8	16.7 ± 0.9	0
Scalp	116 ± 4	121 ± 3	0	33.6 ± 0.6	76.0 ± 2.1	0	23.2 ± 1.0	21.2 ± 1.1	0
Forepaw, palmar region	15 ± 1	40 ± 2	0	1.4 ± 0.0	5.9 ± 0.1	0	33.3 ± 1.2	30.0 ± 1.2	0
Forepaw, dorsum	128 ± 3	130 ± 4	0	21.0 ± 1.0	52.2 ± 2.4	0	20.5 ± 0.5	19.4 ± 0.9	0
Ear	202 + 4	202 ± 3	0	13.5 ± 0.5	13.5 ± 0.8	0	26.1 ± 1.3	26.0 ± 0.9	0
Circumorbital black ring	137 ± 3	142 ± 2	0	8.7 ± 0.8	19.7 ± 1.0	0	18.8 ± 0.9	19.0 + 0.3	0
Eyelid	106 ± 2	106 ± 3	0	5.9 ± 0.1	13.5 ± 0.4	0	24.0 ± 1.1	24.0 ± 1.0	0
Interorbital light streak	29 ± 2	384 ± 5	0	2.5 ± 0.0	72.2 ± 1.8	0	29.2 ± 1.0	22.8 ± 1.0	0
Forehead	39 ± 2	145 ± 4	0	5.7 ± 0.1	90.1 ± 2.1	0	21.9 ± 1.0	17.7 ± 1.0	0
Genital skin, female	378	418	0	26.5	74.5	0	16.9	15.2	9
Genital skin, male	184	175	0	4.9	14.5	0	18.5	19.6	0

[a] TU, 1 tyrosinase unit is the amount of tyrosinase activity required to convert 1 picomole of L-tyrosine to melanin under the conditions of the described assay during a 16 h incubation period at 30 °C; H, homogenate; P, particulate fraction; S, soluble fraction. [b] Specific activity: Specific activity of tyrosinase is the number of TU per μg protein nitrogen. [c] L-tyrosine carboxyl group incorporated into melanin in % of total L-tyrosine converted.

Table II. Inhibitory effects of soluble fractions of *Nycticebus coucang* skin areas on mushroom tyrosinase

Skin (10 mg fresh tissue)	Activity[a] of mushroom tyrosinase (% of control[b])			
	Fresh preparations		3 week preparations	
	H	S	H	S
Forepaw, palm skin	35.2 ± 1.2	33.5 ± 1.0	98.8 ± 1.4	99.1 ± 1.0
Interorbital light streak	13.5 ± 0.7	10.7 ± 0.4	97.3 ± 0.8	101.0 ± 1.2
Forehead	18.9 ± 0.8	15.4 ± 0.7	99.3 ± 1.0	98.8 ± 1.2

[a] 10 µg mushroom tyrosinase was incubated with 40 µg L-tyrosine-^{14}C (sp. act. 0.329 mCi/mmole) in phosphate buffer, 0.1 *M*, pH 6.8, for 1 h at 30 °C. H and S, see footnotes in Table I. [b] Control: activity of mushroom tyrosinase in the absence of homogenates and soluble fractions or in the presence of the corresponding particulate fractions.

Table III. Substrate concentration in the reaction mixture at the beginning and end of incubation with mushroom tyrosinase and with or without soluble fractions of the skin from *Nycticebus coucang*

Origin of soluble fractions (10 mg fresh skin)	Tyrosine concentration (µg/ml)			
	Initial		Terminal	
	No soluble fraction	Soluble fraction	No soluble fraction	Soluble fraction
Forepaw, palm	39.9 ± 0.7	39.5 ± 0.3	36.8 ± 1.4	40.5 ± 1.0
Interorbital light streak	39.9 ± 0.7	40.5 ± 0.5	36.8 ± 1.4	39.4 ± 0.8
Forehead	39.9 ± 0.7	39.9 ± 0.7	36.8 ± 1.4	41.2 ± 0.8

the fraction was derived. Such an increase in tyrosinase activity of the particulate fraction may result from the separation of inhibitors present in the soluble fraction of the homogenate. The in vitro effects of these 3 skin homogenates and their soluble fractions on mushroom tyrosinase supported this thesis (Table II). The inhibitor factor(s) may be reducing agent(s) or substances susceptible to oxidation since skin preparations maintained at 0°–4 °C for 3 weeks lost the ability to inhibit mushroom tyrosinase (Table II). As mushroom tyrosinase incubated in the absence of these skin preparations (Control activity, 100%) was unchanged in the presence of the corresponding particulate fractions, only the soluble fraction appears responsible for the reduction of the tyrosinase activity in the homogenate. To study the 3 soluble fraction preparations which reduce tyrosinase activity, the substrate concentration of mushroom tyrosinase at the beginning and end of incubation was examined in the presence or absence of these fractions (Table III). At the beginning of incubation, the L-tyrosine concentration in the various groups were not different, thereby indicating that little or no free tyrosine was present in the soluble fractions under study. At the end of the incubation period, the L-tyrosine concentration in the absence of the soluble fraction decreased but that in the presence of the soluble fraction was unchanged. Since the tyrosine concentration was determined in the supernates of the TCA precipitated pre- and post-incubation mixtures, the uptake of L-tyrosine by non-tyrosinase reactions in the soluble fraction is eliminated[5].

Résumé. L'analyse radiométrique de la tyrosinase tégumentaire a montré que les régions de la peau de *Nycticebus coucang* diffèrent enzymatiquement. Cette peau renferme un inhibiteur enzymatique de tyrosinase ainsi que des champignons.

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The Carotenoid Pigments of Six Species of Adult Acanthocephala

Adult acanthocephalans are all intestinal parasites of vertebrates and many are coloured orange or red, although the degree of pigmentation varies widely from individual to individual. The orange pigment of *Polymorphus minutus* has been identified as a carotenoid, esterified astaxanthin[1] and carotenoids have also been described from another acanthocephalan *Pallisentis nagpurensis*[2]. In this report the nature of the pigments in 6 species of adult Acanthocephala has been investigated.

Materials and methods. Filicollis anatis were obtained from the common Eider (*Somateria mollissima mollissima*) *Macracanthorhynchus hirudinaceus* from the small intestines of pigs (*Sus scrofa*), *Neoechinorhynchus pseud-*

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