

## CATECHOLAMINES AND RELATED *o*-DIPHENOLS IN THE HEMOLYMPH AND CUTICLE OF THE COCKROACH *LEUCOPHAEA MADERAE* (F.) DURING SCLEROTIZATION AND PIGMENTATION

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**Abstract**—Developmental profiles of catecholamines and related *o*-diphenols in the hemolymph and cuticle of *Leucophaea maderae* were determined during sclerotization and pigmentation of last instar nymphs and adults. *N*-Acetyldopamine (NADA) and dopamine (DA) were the major *o*-diphenols in hemolymph, whereas 3,4-dihydroxyphenylketoethanol (DOPKET), *N*- $\beta$ -alanyldopamine (NBAD), norepinephrine, 3,4-dihydroxyphenylethanol, 3,4-dihydroxyphenylacetic acid, and 3,4-dihydroxyphenylalanine were detected at lower concentrations. The *o*-diphenols occurred primarily as acid-labile conjugates in hemolymph. Dopamine, conjugated as the 3-*O*-sulfate ester, and a NADA conjugate(s) were equal in concentration (0.06 mM) in nymphs shortly before adult apolysis. However, NADA increased after adult ecdysis to a peak at 6 h (0.18 mM), while its precursor DA decreased, suggesting *N*-acetylation of the latter or its metabolism to melanin pigments in the cuticle. In cuticle, NADA, *N*-acetylnorepinephrine (NANE), DOPKET, and *N*- $\beta$ -alanylnorepinephrine (NBANE) accumulated during the early period of adult cuticle sclerotization. DOPKET and NADA (0.4  $\mu$ mol g<sup>-1</sup> each), and NANE (0.2  $\mu$ mol g<sup>-1</sup>) occurred at the highest concentrations in tanned adult cuticle. Large amounts of DOPKET conjugates extracted by cold 1.2 M HCl from tanned cuticle which released DOPKET upon hydrolysis at 100°C for 10 min. DA and NBANE (0.2  $\mu$ mol g<sup>-1</sup> each) predominated in tanned nymphal cuticle. Therefore, sclerotization of nymphal cuticle may require more of the *N*- $\beta$ -alanyl catecholamines, whereas the adult cuticle contains larger quantities of the *N*-acetyl derivatives and ketocatechol (DOPKET) metabolites. Black pigmentation of nymphal and adult cuticle occurs during the first few hours after ecdysis, which correlates with relatively high levels of dopamine.

**Key Word Index:** cockroach, cuticle, *Leucophaea maderae*, sclerotization, catecholamine, dopamine, *N*-acetyldopamine, *N*- $\beta$ -alanyldopamine, hemolymph, *o*-diphenol

### INTRODUCTION

*N*-Acyl catecholamines are the major precursors involved in the sclerotization or tanning of insect cuticle. These and other *o*-diphenols found in tanning structures are derived from the amino acid tyrosine and are oxidized by phenoloxidases to highly reactive quinonoid derivatives that form covalent crosslinks in the chitin-protein matrix of cuticle (reviewed by Brunet, 1980; Lipke *et al.*, 1983; Andersen, 1985; Kramer and Hopkins, 1987). *N*-Acetyldopamine

(NADA) and *N*- $\beta$ -alanyldopamine (NBAD) are synthesized in several species of insects during sclerotization and appear to be the main precursors for tanning agents of new cuticle (Karlson and Sekeris, 1962; Hopkins *et al.*, 1982). They are derived from dopamine (DA) by *N*-acylation with acetate or  $\beta$ -alanine, respectively.

A variety of *o*-diphenolic compounds have been identified in cockroach tissues and cuticle (Czapla *et al.*, 1988). Dopamine-3-*O*-sulfate was first isolated from whole body extracts of newly ecdysed *Periplaneta americana* (Bodnaryk and Brunet, 1974) and is the most abundant catecholamine metabolite present in hemolymph of *P. americana* and some other cockroach species at adult ecdysis (Sloley and Downer, 1987; Czapla *et al.*, 1988). NADA sulfate and phosphate conjugates were identified in tissues of *P. americana* (Bodnaryk *et al.*, 1974). We have determined that NADA instead of DA is the predominant catecholamine in the hemolymph of *Leucophaea maderae* at adult ecdysis (Czapla *et al.*, 1988).

NADA has been associated primarily with stiff colorless cuticle and NBAD with stiff brown cuticle in several insect species (Hopkins *et al.*, 1982, 1984; Kramer *et al.*, 1984; Roseland *et al.*, 1985). However,

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**Abbreviations used:** NADA, *N*-acetyldopamine; DA, dopamine; NBAD, *N*- $\beta$ -alanyldopamine; NANE, *N*-acetylnorepinephrine; NBANE, *N*- $\beta$ -alanylnorepinephrine; NE, norepinephrine; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPET, 3,4-dihydroxyphenylethanol; DOPKET, 3,4-dihydroxyphenylketoethanol; DOBA, 3,4-dihydroxybenzoic acid; DHBA, 3,4-dihydroxybenzylamine; DOPA, 3,4-dihydroxyphenylalanine; AMD,  $\alpha$ -methyldopa; DDC, dopa decarboxylase; LCEC, liquid chromatography with electrochemical detection; TLC, thin-layer chromatography.

in the American cockroach, *P. americana*, both *N*-acetyl- and *N*- $\beta$ -alanyl catecholamines accumulate in high concentrations in tanned cuticle (Czapla *et al.*, 1988). An alternate pathway for DA involves oxidation and cyclization to indole quinones for the production of insect melanins (Hori *et al.*, 1984; Kamer *et al.*, 1983, 1984; Hiruma *et al.*, 1985; Roseland *et al.*, 1987). Since DA serves as a precursor for both the *N*-acyl catecholamines involved in sclerotization and the indole quinone necessary for melanin biosynthesis, the regulation of these pathways is important for species specific coloration and the mechanical properties of the exoskeleton.

*L. maderae* differs from *P. americana* in that black pigmentation of cuticle occurs during the first hours after ecdysis, suggesting melanin deposition in the former. Therefore, we have further investigated the *o*-diphenol precursors that are important for the sclerotization and pigmentation of both nymphs and adults of this cockroach species.

#### MATERIALS AND METHODS

##### Insects

Cockroaches were reared in containers bedded with wood shavings at  $28 \pm 2^\circ\text{C}$  with a photoperiod of 16L:8D. Water and Purina® "Lab Chow" were provided *ad lib*. Last stage nymphs were placed in individual rearing cages, and the ages of individual insects were determined from the last nymphal or adult ecdysis. Pharate stages were timed using eye color as an indicator (Czapla *et al.*, 1988).

##### Hemolymph extraction

Cockroaches were anesthetized with carbon dioxide, quick-frozen in dry ice powder and stored at  $-20^\circ\text{C}$  until analyzed. After thawing in a desiccator for 5 min, the insects were placed in a spring steel clip that applied light pressure to the abdomen. The insect was suspended head down for 1 min, the coxa of a front leg was severed, and hemolymph was collected with a microcapillary pipette. This was diluted 10-fold in 1.2 N HCl containing 5 mM ascorbic acid and  $6 \mu\text{g ml}^{-1}$  of either 3,4-dihydroxybenzylamine (DHBA) or  $\alpha$ -methyl dopa (AMD) as an internal standard. The extracts were centrifuged at 6500 *g* for 10 min, and the supernatant was removed for analysis by LCEC (Czapla *et al.*, 1988).

##### Cuticle extraction

The pronotum was dissected and placed in distilled water containing a few crystals of phenylthiourea. Adhering muscle and fat body were teased away from the cuticle, and the inner surface was scraped and rinsed with distilled water to remove epidermis. Pieces of cuticle were blotted dry on absorbent tissue, weighed, and homogenized in ground glass tissue grinders in 0.3 ml of ice cold 1.2 M HCl, 5 mM ascorbate containing 0.12  $\mu\text{g}$  DHBA or 1.2  $\mu\text{g}$  AMD. The homogenate was centrifuged at 6500 *g* for 10 min, and the supernatant was removed for LCEC analysis (Czapla *et al.*, 1988). In some instances the supernatant was hydrolyzed (10 min at  $100^\circ\text{C}$ ) before alumina recovery (Morgan *et al.*, 1987) and in other cases the supernatant was recovered from alumina and then hydrolyzed.

##### Analysis of *o*-diphenols

Aliquots of the 1.2 M HCl hemolymph supernatants were heated at  $100^\circ\text{C}$  under nitrogen for 10 min to release *o*-diphenols from their acid labile conjugates. Samples of both hydrolyzed and unhydrolyzed extracts were adsorbed on alumina, and the *o*-diphenols were recovered in 1 M acetic acid for LCEC analysis. The mobile phases used for analysis of hemolymph (Fig. 1A) and cuticular samples

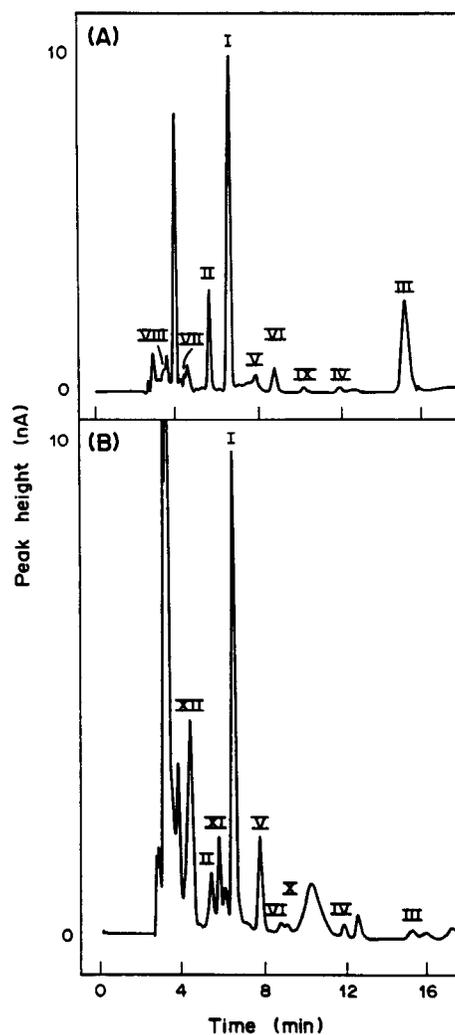


Fig. 1. LCEC chromatograms of extracts from *Leucophaea maderae* adults. (A) Hemolymph 6 h after ecdysis and (B) cuticle 24 h after ecdysis. I:AMD (internal standard); II:DA; III:NADA; IV:NBAD; V:DOPKET; VI:DOPET; VII:DOPA; VIII:NE; IX:DOPAC; X:DOBA; XI:NANE; XII:NBANE. See Materials and Methods for details of the mobile phases.

(Fig. 1B) have been described previously (Czapla *et al.*, 1988). Retention times of *o*-diphenolic standards were compared to unknown electroactive peaks in the extracts using both mobile phases. Quantities of individual *o*-diphenols were calculated by comparing peak heights with that of the internal standard in each extract and then correcting for recoveries established using standard compounds. The percent conjugation of each compound was calculated from the amounts present before and after hot acid hydrolysis. Chemical standards were obtained from commercial sources or were synthesized (Hopkins *et al.*, 1982; Morgan *et al.*, 1987). Two-dimensional mapping of radiolabeled tyrosine or sulfate metabolites by TLC and electrophoresis were conducted as described previously (Czapla *et al.*, 1988).

#### RESULTS

##### *o*-Diphenols in hemolymph

NADA was the major *o*-diphenol at adult ecdysis and during cuticle tanning, and lesser amounts (in

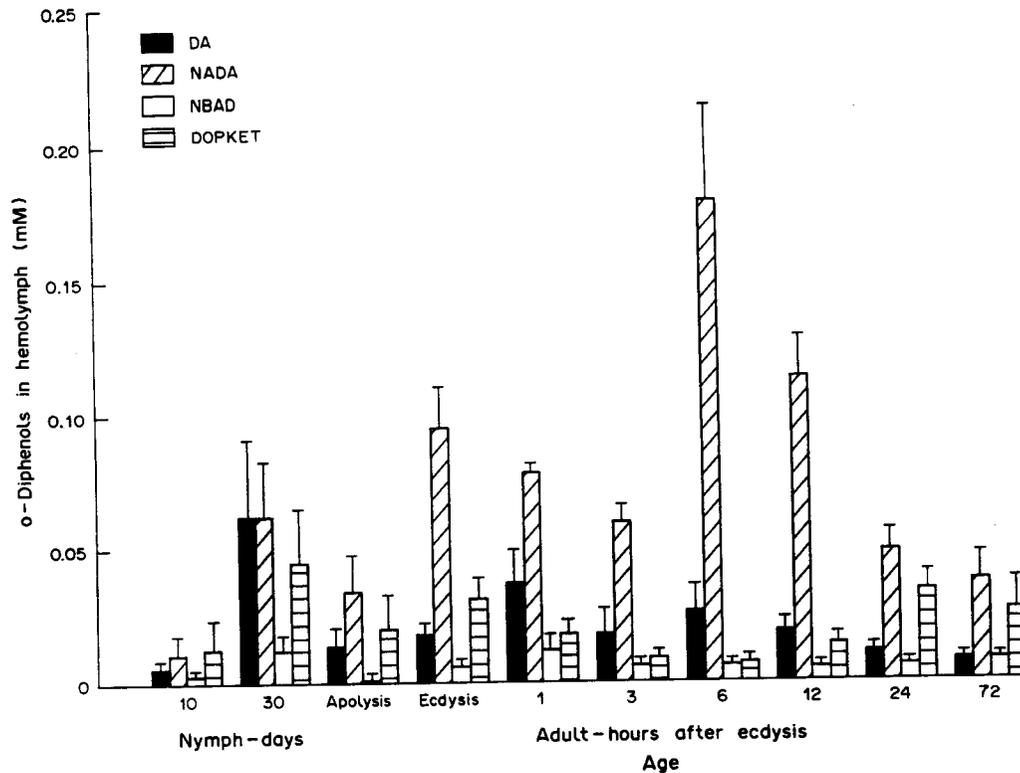


Fig. 2. Concentrations of major *o*-diphenols in hemolymph (hydrolyzed) of *Leucophaea maderae* during last nymphal stadium, adult ecdysis, and cuticle tanning. Data are means of 3–8 animals  $\pm$  SEM.

decreasing order) of DA, DOPKET, NE, DOPET, DOPA, NBAD and DOPAC were also present (Figs 1A and 2). During the last nymphal stadium, NADA levels increased by 10-fold from 0.01 mM in 10-day nymphs to 0.10 mM at adult ecdysis. However, peak levels occurred 6 h later (0.18 mM) and then declined to about 0.05 mM as the cuticle darkened and sclerotized by 24 h. Approximately 50–80% of NADA in the hemolymph was sequestered as an acid-labile conjugate(s) (Table 1). The largest percentage of free NADA occurred in newly ecdysed insects.

DA concentration in hemolymph increased over 10-fold during the last nymphal stadium, reaching peak levels (0.06 mM) just before apolysis to pharate adults (Fig. 2). A 6-fold decrease occurred at ecdysis, followed by a moderate increase 1 h later (0.04 mM). DA then declined to low levels during the period of cuticle sclerotization. DA was present as an acid-labile conjugate, which we have identified as the

3-O-sulfate ester (see Conjugate Identification section). Over 70% of DA was conjugated with sulfate in 10-day nymphs, and this increased to almost 100% at the end of the stadium. The percentage of conjugated DA decreased in pharate and newly ecdysed adults, but again increased after the first few hours of sclerotization (Table 1).

DOPKET levels increased 6-fold during development of the last instar to 0.04 mM in the hemolymph of 30-day nymphs (Fig. 2). This compound decreased in concentration during cuticle tanning, and then gradually returned to pre-ecdysial levels 3 days later. DOPKET was present primarily as an acid-labile conjugate(s); only trace levels of free DOPKET were detected in unhydrolyzed hemolymph extracts. The other *o*-diphenols identified in hydrolyzed hemolymph extracts (NBAD, DOPA, NE, DOPAC, and DOPET) were less than 0.02 mM during both the last nymphal period and adult cuticle sclerotization.

Table 1. Percent conjugation of *o*-diphenols in the hemolymph of *Leucophaea maderae*\*

Stage	Age after ecdysis	DA	NADA	NBAD	DOPKET
Nymph last instar	Days				
	10	73 $\pm$ 8	53 $\pm$ 12	18 $\pm$ 4	100
	30	97 $\pm$ 1	68 $\pm$ 9	55 $\pm$ 12	100
Pharate adult	Apolysis	46 $\pm$ 15	80 $\pm$ 4	ND	ND
Adult	Hours				
	0 (ecdysis)	61 $\pm$ 15	56 $\pm$ 6	ND	100
	3	56 $\pm$ 23	83 $\pm$ 1	73 $\pm$ 14	ND
	6	86 $\pm$ 10	70 $\pm$ 10	85 $\pm$ 9	100
	24	85 $\pm$ 2	83 $\pm$ 1	67 $\pm$ 22	97 $\pm$ 3
	72	72 $\pm$ 3	83 $\pm$ 1	58 $\pm$ 13	100

\*Data are means of 3–5 samples  $\pm$  SEM. ND = Not determined.

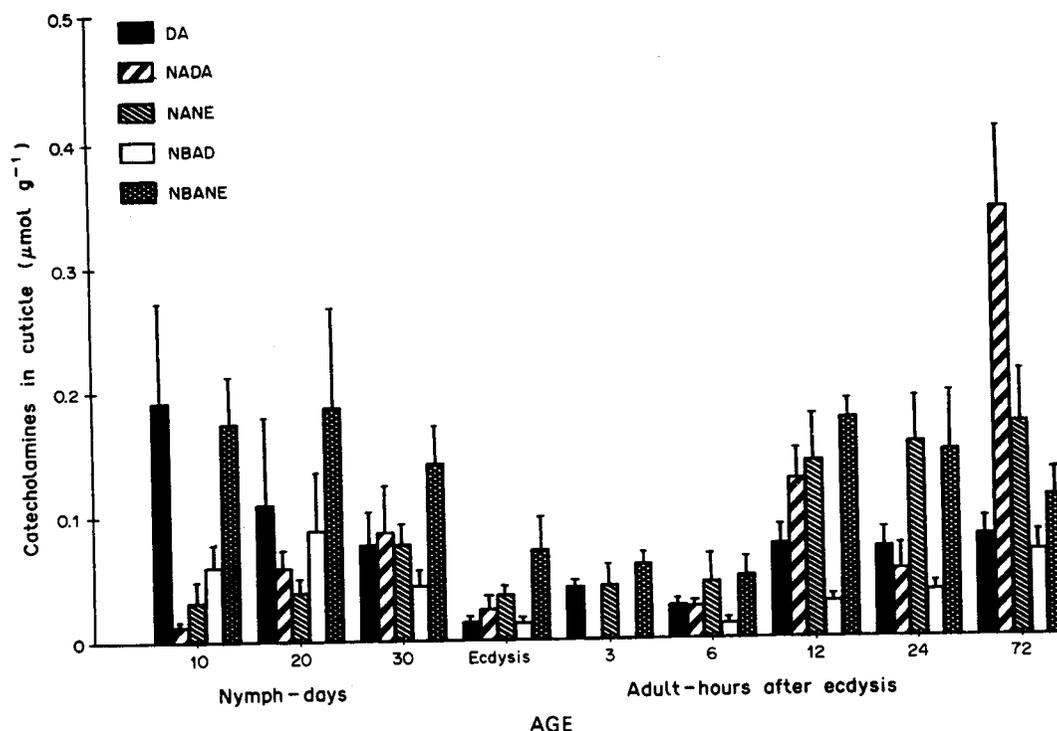


Fig. 3. Concentrations of catecholamines in cuticle (cold-acid extracts) of *Leucophaea maderae* during last nymphal stadium, adult ecdysis, and cuticle tanning. Data are the means of 3–6 samples  $\pm$  SEM.

#### *o*-Diphenols in cuticle

At adult ecdysis, NBANE was the major diphenol present in *L. maderae* cuticle ( $0.07 \mu\text{mole g}^{-1}$ ), whereas other diphenols were detectable at lower concentrations (Fig. 3). By 3 h after ecdysis, NANE and DA had increased to levels nearly comparable to that of NBANE, while NADA and NBAD concentrations were less than  $0.01 \mu\text{mole g}^{-1}$ . NBANE reached peak levels at 12 h ( $0.18 \mu\text{mole g}^{-1}$ ) and gradually declined to nearly half that amount by 72 h. NANE increased approximately 5-fold between ecdysis and reached peak levels at 72 h ( $0.17 \mu\text{mole g}^{-1}$ ), whereas DA increased only slightly ( $0.08 \mu\text{mole g}^{-1}$ ). NADA concentrations were low during the first few hours after ecdysis, but rapidly increased by 12 h ( $0.13 \mu\text{mole g}^{-1}$ ) and continued to rise, reaching peak levels by 72 h ( $0.34 \mu\text{mole g}^{-1}$ ), when NADA became the major acid extractable catecholamine in

*L. maderae* cuticle. NBAD also attained a peak level at 72 h ( $0.07 \mu\text{mole g}^{-1}$ ), but its concentration was 5 times less than that of NADA. DOPKET levels were low during the initial period of adult sclerotization ( $0.03 \mu\text{mole g}^{-1}$ ), but increased almost 6-fold by 12 h and continued to increase to a peak level at 72 h ( $0.39 \mu\text{mole g}^{-1}$ ; Table 2). Other *o*-diphenols detected in minor concentrations were DOPAC, DOPET, and DOBA. Their concentrations remained relatively constant during cuticular sclerotization (Table 2). DOPKET concentrations ( $2.8 \pm 0.2 \mu\text{mole g}^{-1}$ ) were extremely high in 24 h hydrolyzed cuticle extracts, some 10 times greater than unhydrolyzed extracts whereas NADA increased ( $0.3 \mu\text{mole g}^{-1}$ ) approximately 3-fold. An increase in DOPKET ( $0.61 \mu\text{mole g}^{-1}$ ) was also observed after hydrolyzing alumina recovered extracts, whereas NADA concentrations did not increase.

Table 2. Concentrations of *o*-diphenols with acid or alcohol side chains ( $\mu\text{mole g}^{-1}$ ) in cuticle (cold-acid extracts) of *Leucophaea maderae* during the last-nymphal stadium and adult cuticle tanning\*

Stage	Age after ecdysis	DOPKET	DOPET	DOPAC	DOBA
Nymph	Days				
	10	$0.06 \pm 0.02$	$0.04 \pm 0.01$	0.01	trace†
	20	$0.05 \pm 0.02$	$0.03 \pm 0.03$	0.01	trace
	30	$0.06 \pm 0.01$	$0.04 \pm 0.01$	0.01	trace
Adult	Hours				
	0 (ecdysis)	$0.03 \pm 0.02$	$0.05 \pm 0.03$	$0.03 \pm 0.02$	0.01
	3	0.01	trace	0.01	0.01
	6	$0.02 \pm 0.01$	$0.03 \pm 0.01$	0.01	0.01
	12	$0.23 \pm 0.04$	$0.02 \pm 0.01$	0.01	0.01
	24	$0.21 \pm 0.09$	0.01	trace	trace
	72	$0.39 \pm 0.09$	$0.07 \pm 0.04$	$0.02 \pm 0.01$	0.01

\*Data are the means of 3–6 samples  $\pm$  SEM where applicable.

†Values  $< 0.01 \mu\text{mole g}^{-1}$  are labeled as trace.

Cuticle of the last nymphal instar displayed a different *o*-diphenol profile than that of adult cuticle (Fig. 3). DA and NBANE predominated in 10-day tanned nymphal cuticle, rather than the *N*-acetyl catecholamines and DOPKET that were major in adult cuticle. DA declined during the nymphal period, whereas NBANE concentrations remained constant. NADA was extremely low at 10 days ( $0.01 \mu\text{mole g}^{-1}$ ), but it increased 9-fold by 30 days. NANE, NBAD, and DOPKET in nymphal cuticle displayed little variation in concentration (Fig. 3, Table 2).

The total cuticular concentration of the *N*-acetyl catecholamines, NADA and NANE, were equivalent to or greater than that of DOPKET during the period of sclerotization. The cuticle concentrations of the *N*- $\beta$ -alanyl catecholamines were about 25% less than those of the *N*-acetyl catecholamines at 12 and 24 h and 50% less at 72 h (Fig. 3, Table 2).

#### Conjugate identification

We have confirmed the identity of dopamine-3-O-sulfate in the hemolymph of *L. maderae*. Electrophoretic and thin-layer chromatographic (TLC) separations of hemolymph extracts from cockroaches injected with either [ $^{14}\text{C}$ ]tyrosine or [ $^{35}\text{S}$ ]sulfate revealed the presence of a major metabolite labeled with  $^{14}\text{C}$  or  $^{35}\text{S}$  and having mobility identical to that of dopamine sulfate (Czapla *et al.*, 1988). After recovery of this metabolite from cellulose TLC plates and acid hydrolysis, LCEC analysis revealed DA to be the tyrosine metabolite. When the conjugate was recovered and analyzed by LCEC without prior hydrolysis, it coeluted with dopamine 3-O-sulfate. The identity of the NADA and DOPKET hemolymph conjugates were not determined.

#### DISCUSSION

*N*-Acetyldopamine and DA are the major catecholamines in hemolymph of last instar nymphs and adults of *L. maderae*. NADA and DA occur primarily as acid-labile conjugates, the latter as the 3-O-sulfate ester. Dopamine-3-O-sulfate is also the major *o*-diphenol in *P. americana* during cuticle tanning (Bodnaryk and Brunet, 1974; Sloley and Downer, 1987; Czapla *et al.*, 1988). The pattern of accumulation of catecholamines in hemolymph during the last nymphal stadium of *L. maderae* is similar to that observed in *P. americana* (Czapla *et al.*, 1988). Both DA and NADA conjugates increase in the hemolymph during the last nymphal stadium, with DA reaching maximal levels in the pharate adult shortly before ecdysis. DA sulfate then decreases in newly ecdysed adults, whereas NADA reaches peak levels 6 h after adult ecdysis.

The sequestration of catecholamine conjugates during the feeding period of nymphal cockroaches for later use in the sclerotization and melanization of cuticle differs from processes in the tobacco hornworm, *M. sexta*, and possibly other lepidopterous and dipterous insects. In *M. sexta*, catecholamine conjugates accumulate only in the hemolymph after apolysis to the pharate stage. However, tyrosine conjugates, including tyrosine glucoside and tyrosine phosphate, appear to be more commonly sequestered

during the larval feeding period of Lepidoptera and Diptera (Kramer and Hopkins, 1987).

Developmental profiles of enzyme activities in the catecholamine pathway indicate that low levels of synthesis could account for the accumulation of the catecholamine conjugates during nymphal feeding. DOPA decarboxylase activity (DDC) is high at nymphal ecdysis of *L. maderae* and then maintains a low level during the nymphal feeding period (Wirtz and Hopkins, 1977a). Free tyrosine concentrations also decrease after nymphal ecdysis, but then gradually increase during the stadium (Wirtz and Hopkins, 1977b). A low level of tyrosine hydroxylase activity could account for the synthesis of DOPA (Hopkins, 1982), which is then metabolized by DDC, dopamine *N*-acyltransferase and conjugating enzymes in the catecholamine storage molecules found in *L. maderae* and *P. americana* (Czapla *et al.*, 1988). A major difference between the two species is the much higher levels of DA 3-O-sulfate and NADA conjugate(s) in *P. americana*. Except for *L. maderae*, in which NADA predominates, DA is also the most abundant *o*-diphenol in hemolymph at adult ecdysis in several other cockroach species (Czapla *et al.*, 1988).

Even though catecholamines are sequestered in last instar nymphs, a large amount of catecholamine biosynthesis appears to occur in the newly ecdysed adult. Free tyrosine reaches peak levels in *L. maderae* at adult ecdysis but it rapidly declines during the next 24 h as sclerotization and melanization of cuticle occur (Wirtz and Hopkins, 1977a,b). This would indicate a high rate of hydroxylation to DOPA by a tyrosine hydroxylase or tyrosinase enzyme, as was shown with *P. americana* (Hopkins, 1982). Since DOPA never accumulates to significant levels in the hemolymph and cuticle, it must be rapidly decarboxylated to DA. Both *in vivo* and *in vitro* studies show high levels of DDC activity after adult ecdysis of *L. maderae* (Wirtz and Hopkins, 1977b). Although DA production is high during cuticle sclerotization, its concentrations decrease in hemolymph and show only small increases in the cuticle. Much of this DA may be utilized for melanin biosynthesis, which mainly occurs in *L. maderae* during the first few hours following ecdysis. However, NADA concentrations increase to peak levels after ecdysis in both hemolymph and cuticle, suggesting a high level of dopamine-*N*-acetyl transferase activity. The subsequent decline of NADA in hemolymph after 6 h correlates with its transport into the tanning cuticle, as cuticle concentrations continue to increase through 72 h.

*o*-Diphenol concentrations in the cuticle of newly ecdysed adults are relatively low, with *N*- $\beta$ -alanyl-norepinephrine (NBANE) having the highest level. However, between 6 and 12 h postecdysis, the concentrations of DA, NADA, NANE, NBANE, and DOPKET increase, while that of NBAD, the precursor of NBANE, remains relatively constant. Between 1 and 3 days, NADA and DOPKET reach very high concentrations ( $0.4 \mu\text{moles g}^{-1}$ ). The oxidation of these compounds in cuticle by phenoloxidases could produce quinonoid derivatives for sclerotization. These highly reactive compounds can form adducts or crosslinks via covalent bonds between ring or side chain carbons of the catecholamines to nucleophilic

groups of cuticular proteins (Andersen, 1985; Lipke *et al.*, 1983; Schaefer *et al.*, 1987; Kramer and Hopkins, 1987).

The stabilization of *L. maderae* cuticle involves both the *N*-acetyl and *N*- $\beta$ -alanyl dopamines and norepinephrines, as was found with *P. americana* (Czapla *et al.*, 1988). However, *L. maderae* appears to utilize the *N*-acetyl catecholamines more extensively than the *N*- $\beta$ -alanyl derivatives, whereas the opposite was found in *P. americana*. The predominance of NADA in the hemolymph of *L. maderae* correlates with the high levels of NADA and NANE in the cuticle. This is quite different from *P. americana*, in which NBAD is not sequestered in the hemolymph, despite the presence of high levels of both NBAD and NBANE in the cuticle. Therefore, *N*- $\beta$ -alanylation of DA may occur in the epidermis with transport mainly in the direction of the cuticle where oxidation of NBAD to NBANE occurs (Morgan *et al.*, 1987). The *N*-acetylation of DA to NADA takes place in other tissues, such as the fat body, where conjugation may also occur. NADA conjugates then would be released into the hemolymph.

The abundance of DOPKET in cold-acid extracts of *L. maderae* cuticle was unexpected. DOPKET is usually released from dimeric compounds in cuticle by hot acid hydrolysis and is indicative of the occurrence of  $\beta$ -sclerotization in cuticle (Andersen, 1971; Andersen *et al.*, 1980; Andersen and Roepstorff, 1981). The large amounts of DOPKET released upon hydrolysis of the cold acid extracts before and after alumina recovery supports the hypothesis that some DOPKET is present in *L. maderae* cuticle as soluble conjugates or dimer-like compounds. DOPKET released upon hydrolysis is probably not derived from the NADA dimer since the ratio of DOPKET to NADA in the hydrolysate is not equivalent (Andersen *et al.*, 1980). However, DOPKET may be released from a dimer consisting of DOPKET and NADA (Andersen and Roepstorff, 1981). Some of the DOPKET in the cold-acid extract may be released during homogenization. However, free DOPKET appears to exist in sclerotized cuticle, since it has been extracted by hot-water from *P. americana* exuviae (Atkinson *et al.*, 1973) and by 80% methanol from *P. americana* cuticle (Morgan *et al.*, 1987). It has been proposed that DOPKET and other diphenols with acid, aldehyde, or alcohol sidechains may function as epicuticular lipid antioxidants (Atkinson and Gilby, 1970; Atkinson *et al.*, 1973). However, the relatively high levels of DOPKET conjugates in the cuticle and hemolymph of *L. maderae* indicate that it is involved in the sclerotization of the exoskeleton.

Cuticular coloration of insects appears to depend to some extent upon the types of catecholamines transported into cuticle (Hopkins *et al.*, 1982, 1984). In the clear or lightly colored cuticle with black melanic markings of *L. maderae*, the *N*-acetyl diphenols, NADA and NANE, along with DOPKET, are the primary *o*-diphenols present, whereas the reddish brown cuticle of *P. americana* contains higher levels of the *N*- $\beta$ -alanyl catecholamines, NBAD and NBANE. These data support the hypothesis that the *N*-acetyl catecholamines are more involved in the sclerotization of hard clear cuticle, whereas the

*N*- $\beta$ -alanyl catecholamines stabilize hard dark cuticle (Hopkins *et al.*, 1984). Dopamine has been shown to be a precursor of insect melanins (Hori *et al.*, 1984; Hiruma *et al.*, 1985). Melanization occurs in *L. maderae* cuticle during the first few hours after ecdysis, which correlates with the relatively high DA concentrations. Inhibition of DDC immediately after ecdysis prevents the black pigmentation of *L. maderae* cuticle, which indicates that dopamine melanins are responsible for the black pigments in this species. However, sclerotization continues slowly over several days in these insects, as the cuticle becomes a light yellowish color (Hopkins, unpublished data). *L. maderae* cuticle undergoes melanization whereas *P. americana* cuticle does not appear to melanize, even though it contains equivalent or higher concentrations of DA. However, DA represents a much larger percentage of the extractable catecholamines during the same period in *L. maderae* cuticle than in *P. americana* cuticle.

Sclerotization and pigmentation of *L. maderae* cuticle appear to involve a complex pattern of *o*-diphenol biosynthesis to supply the necessary quinonoid compounds for melanin biosynthesis and cuticle stabilization. The regional utilization of these metabolites probably depends upon the functional properties of the exoskeleton. *L. maderae* accumulates both *N*-acetyl and *N*- $\beta$ -alanyl catecholamines in tanning cuticle, but patterns differ from those of *P. americana*. Unlike *P. americana*, which has a red-brown cuticle, *L. maderae* deposits melanin in a species specific pattern and simultaneously sclerotizes the cuticle into a clear or lightly colored sclerotin masked by black pigmentation.

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