Mass spectra of the scent scale substances of *P. melete* and *P. napi*

Peak No.	Component	R_t	M^+	H_1	H_2	H_3	*P. melete* (%)	*P. napi* (%)
1	Unknown	0.60	104	104	78	43	5.0	2.1
2	α-Pinene	1.00	136	93	77	41	8.7	5.4
3	β-Pinene	1.28	136	93	41	69	4.0	3.0
4	Myrcene	1.44	136	41	93	69	4.3	4.2
5	p-Cymene	1.72	134	119	134	91	3.7	4.3
6	Limonene	1.87	136	68	93	67	3.6	3.6
7	Linalool	2.88	154	71	41	93	–	8.8
8	n-Undecane	3.11	156	57	43	71	4.0	1.1
9	Neral	5.27	152	69	41	94	35.4	44.3
10	Geranial	5.72	152	69	41	84	31.1	23.2

R_t: α-pinene = 1.00, M^+; molecular ion, H_1; base ion, H_2; 2nd-highest ion, H_3; 3rd-highest ion.

Results. The results are shown in the table and the figure. 7 monoterpenes (α-pinene, β-pinene, myrcene, p-cymene, limonene, neral and geranial) were identified together with n-undecane from the scent scales of *P. melete*, while these 7 and considerable amounts of linalool were identified in the scent scales of *P. napi* (figure). Content ratio of neral to geranial in *P. melete* was 0.77–1.04 and that in *P. napi* was 1.84–2.43. Although it is difficult to distinguish the 2 species, since morphological differences between them are slight, the present results suggest them to be tentatively identified by the ratio of neral to geranial and the presence or absence of linalool in the scent scales.

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The association between N-acetyl-neuraminic acid and calcium ions in aqueous solution

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Summary. The conductances of aqueous solutions of the calcium salt of N-acetyl-neuraminic acid (NANA) an important constituent of glycolipids and glycoproteins have been measured over a range of temperature. The data are consistent with the formation of the $Ca(NANA)^+$ ion-pair. Dissociation constants and thermodynamic parameters for the ion-pair have been derived.

N-acetyl-neuraminic acid (NANA) is an important constituent of membrane glycolipids and glycoproteins and it has been shown to be responsible for a large proportion of the surface charge of erythrocytes[1]. The interaction of calcium ions with NANA has been implicated in a variety of physiological and biochemical processes[2-4]. Behr and Lehn[5] investigated the interaction between alkali and alkaline-earth cations using ion selective electrodes and the binding of calcium ions at high concentrations (0.1-4 M) to NANA and its derivatives has been studied using ^{13}C NMR[6,7].

In this communication a conductimetric study of the calcium salt of NANA in aqueous solution over a range of temperature is reported. Conductivity measurements afford a sensitive means of detecting ion-pair formation at physiological Ca^{2+} ion concentrations. The data show that there are considerable deviations from the Onsager limiting law for conductivity. The results can be interpreted in terms of the formation of the $Ca(NANA)^+$ ion-pair for which the dissociation constant and thermodynamic parameters are derived.

Synthetic 'pure' NANA was obtained from Koch-Light Laboratories Ltd., and was used as supplied. Aqueous solutions of the calcium salt were prepared by conductimetric titrations of standardized calcium hydroxide solutions (nominally 6 mM) with NANA solutions (nominally 12 mM). The conductivities were measured in the range 0.1–2.5 mM using specially constructed

Table 1. Calculation of dissociation constants for the $Ca(NANA)^+$ ion-pair from conductance measurements at 25 °C

Molarity (moles/l) × 10^3	Λ_e ($cm^2\ \Omega^{-1}\ equiv^{-1}$)	$[Ca(NANA)^+] \times 10^3$ (moles/l)	$I \times 10^3$	f*	$K_{diss} \times 10^3$ (moles/l)
2.768	70.65	1.157	5.99	0.6944	4.241
2.666	70.71	1.118	5.76	0.6994	4.076
1.596	79.57	0.4975	3.79	0.7482	4.452
0.9108	87.48	0.1988	2.33	0.7964	4.628
0.9072	87.80	0.1937	2.33	0.7964	4.754
0.4112	94.84	0.06063	1.11	0.8550	3.718
0.4088	94.16	0.05668	1.12	0.8541	4.092
0.2316	98.51	0.02304	0.65	0.8869	3.536

* Calculated from $\log f\pm = -4 \times 0.5115\sqrt{I}$.

Table 2. Parameters derived from the conductance of the calcium salt of N-acetyl-neuraminic acid, concentration range 0.1-2.5 mM

Temperature (°C)	$\lambda^{\circ}_{Ca^{2+}}$* ($cm^2\,\Omega^{-1}\,equiv^{-1}$)	$\lambda^{\circ}_{NANA^-}$ ($cm^2\,\Omega^{-1}\,equiv^{-1}$)	% Ion-pairs** at 2.5 mM	$K_{diss} \times 10^3$*** (moles/l)	ΔG° (kJ $mole^{-1}$)	ΔS° (J $mole^{-1}\,K^{-1}$)
0	31.2	28.0	62.0	2.03 ± 0.18	13.1	26.6
25	59.5	49.3	39.5	4.19 ± 0.40	13.6	25.1
37	74.5	56.3	30.7	6.12 ± 0.55	14.1	25.6

*Robinson and Stokes[8], p.465; **(x/M)× 100; *** ± SD.

dilution cells of low cell constant (1-1.5 cm^{-1}) with platinum electrodes. The cells had a minimum capacity ~10 cm^3. They were thermostatted in an oil-bath and conductances were measured with a Wayne Kerr Universal Bridge B 224. Dilutions were carried out by weight and the results were corrected for the conductance of the doubly-distilled water used (specific conductance $\sim 3 \times 10^{-6}\ \Omega^{-1}\ cm^{-1}$ at 25 °C).

The figure 1 shows the plots of the equivalent conductances (Λ_e) as a function of the square root of the molarity (M) of NANA. Treatment of these data by the method of least squares gives the following equations for Λ_e.

$$\begin{aligned} \Lambda_e\ (0\,°C) &= 59.2\text{-}536.4\sqrt{M} \\ \Lambda_e(25\,°C) &= 108.8\text{-}725.7\sqrt{M} \\ \Lambda_e(37\,°C) &= 130.8\text{-}760.4\sqrt{M} \end{aligned} \qquad (1)$$

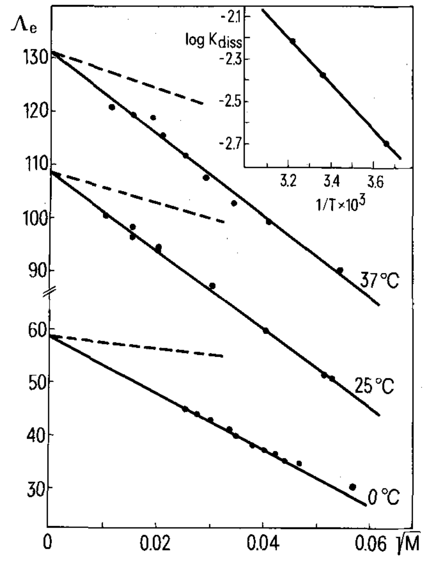
According to the Onsager limiting law for the equivalent conductance[8] of a 2:1 electrolyte of molarity M,

$$\Lambda_e = \Lambda_o - \left[\frac{5.602 \times 10^6 q \Lambda_o}{(\varepsilon_r T)^{3/2}(1+\sqrt{q})} + \frac{123.75}{\eta(\varepsilon_r T)^{1/2}}\right] \times 1.732\sqrt{M} \qquad (2)$$

where ε_r is the relative permittivity, T is the absolute temperature, η is the viscosity of water, and

$$q = \frac{2}{3}\left[\frac{\lambda^{\circ}_{Ca^{2+}} + \lambda^{\circ}_{NANA^-}}{2\lambda^{\circ}_{NANA^-} + \lambda^{\circ}_{Ca^{2+}}}\right]$$

where $\lambda^{\circ}_{Ca^{2+}}$ and $\lambda^{\circ}_{NANA^-}$ are the limiting equivalent ionic conductances of the Ca^{2+} and $NANA^-$ anion respectively. Values of $\lambda^{\circ}_{NANA^-}$ were obtained from the experimental equivalent conductances at infinite dilution Λ_o and the literature values of $\lambda^{\circ}_{Ca^{2+}}$ (table 2). The dashed lines in the figure were calculated by substitution of the appropriate parameters into equation (2). It is clear that there are considerable deviations between the experimentally obtained lines and those calculated from the Onsager equation. Furthermore plots of $\Lambda_e + S\sqrt{M}$ (not shown), where S is the gradient given by the Onsager equation, against M have negative gradients which is a criterion for ion-pairing[9].



Equivalent conductance $\Lambda_e(\Omega^{-1}\ cm^2\ equiv^{-1})$ as a function of $(molarity)^{1/2}$ for the calcium salt of N-acetyl-neuraminic acid in aqueous solution. The dotted lines were calculated from the Onsager equation. The inset shows the plot of $\log K_{diss}$ against $10^3\ T^{-1}$ for the $Ca(NANA)^+$ ion-pair.

The results can be interpreted in terms of the following equilibrium between the ion-pair $Ca(NANA)^+$ and the corresponding free ions.

$$Ca(NANA)^+ \rightleftharpoons Ca^{2+} + NANA^- \qquad (3)$$

If x is the molar concentration of $Ca(NANA)^+$ and M is the total molarity of $Ca(NANA)_2$ then

$$K_{diss} = \frac{[Ca^{2+}][NANA^-]f}{[Ca(NANA)^+]} = \frac{(M-x)(2M-x)f}{x} \qquad (4)$$

Where f is the activity coefficient of the Ca^{2+} ion which can be calculated from the Debye-Hückel expression $\log f = -4A\sqrt{I}$ where the ionic strength $I = 3M - 2x$ and A is the Debye-Hückel constant. Note the activity coefficients of the $Ca(NANA)^+$ and $NANA^-$ ions cancel.

The application of conductivity measurements to the study of ion-pairing has been discussed by Jenkins and Monks[10] and Davies[11]. Following the precedure of Jenkins and Monks the equivalent conductance of the solution is given in terms of the $Ca(NANA)_2$ concentration in equivalents per l (C = 2 M) by

$$\Lambda_e C = x\Lambda_{Ca(NANA)^+} + (C-2x)\Lambda_{Ca^{2+}} + (C-x)\Lambda_{NANA^-}$$

$$\therefore \Lambda_e = \Lambda_{Ca^{2+}} + \Lambda_{NANA^-} + \frac{x}{C}(\Lambda_{Ca(NANA)^+} - 2\Lambda_{Ca^{2+}} - \Lambda_{NANA^-}) \qquad (5)$$

In order to apply equation (5) to calculate x as a function of M it is necessary to have equations for $\Lambda_{Ca^{2+}}$, Λ_{NANA^-} and $\Lambda_{Ca(NANA)^+}$. The equivalent conductances of the Ca^{2+} and $NANA^-$ ions can be expressed by the appropriate Onsager equations, thus at 25 °C

$$\begin{aligned} \Lambda_{Ca^{2+}} &= 49.3 - 108.1\sqrt{M} \\ \Lambda_{NANA^-} &= 59.5 - 130.4\sqrt{M} \end{aligned}$$

For $\Lambda_{Ca(NANA)^+}$ we have taken ½ that of $\Lambda_{Ca^{2+}}$. i.e.

$$\Lambda_{Ca(NANA)^+} = 29.8\text{-}65.2\sqrt{M}$$

As pointed out by Davies[9] the value assigned to the ion-pair is not critical. Thus if $\Lambda_{Ca(NANA)^+}$ is taken as ⅔ $\Lambda_{Ca^{2+}}$ the effect on the calculated values of the equilibrium constant is very small. By this procedure values of x were obtained using equation (5) and the observed values of Λ_e. The values of K_{diss} were calculated from equation (4). Some specimen results at 25 °C are shown in table 1.

Table 2 gives the values of K_{diss} as a function of temperature together with derived thermodynamic parameters. Using an ion selective electrode a value of K_{diss} = 13 mM at 25 °C was found by Behr and Lehn[5] (ionic strength 0.02-0.04) and Jacques et al.[7] quote values of 8.26 ± 0.2 mM using calcium ion activity coefficients derived from calcium chloride solutions and 4.39 mM assuming calcium ion activities of unity. The present method gives a result in reasonable agreement with the latter value. Dissociation of the $Ca(NANA)^+$ ion increases markedly with temperature; at a total calcium ion concentration of 2.5 mM, 30.7% of the calcium is complexed to $NANA^-$ at 37 °C. The inset in the figure shows the plot of $\log K_{diss}$ as a function of reciprocal temperature, the slope of this plot is $-\Delta H°/2.303\,R$ (Gibbs-Helmholtz equation). The enthalpy of dissociation ($\Delta H°$) is constant at 21.1 kJ $mole^{-1}$. It is clear that at physiological calcium levels the conductance method shows conclusively that NANA binds Ca^{2+} ions.

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26-Hydroxy-β-ecdysone, a metabolite of β-ecdysone in the blowfly, *Calliphora erythrocephala*

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Summary. 26-Hydroxy-β-ecdysone has been identified as a metabolite of the moulting hormone, β-ecdysone in *Calliphora erythrocephala.*

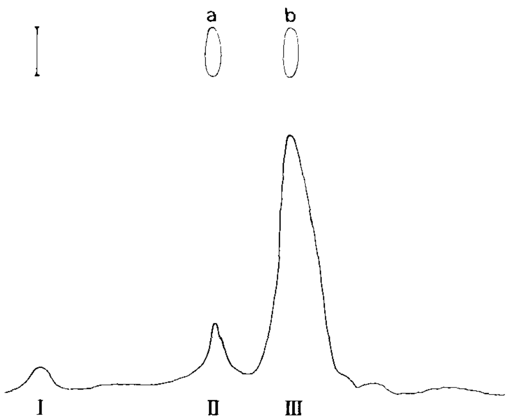
26-Hydroxy-β-ecdysone has been isolated as one of the insect moulting hormones present in the tobacco hornworm, *Manducca sexta*[1-3], and it has also been identified as a metabolite of synthetic 22,25-dideoxy-α-ecdysone[4-6] in this insect. In studies on the metabolism of tritiated α- and β-ecdysone in *Calliphora erythrocephala*[7,8], *Locusta migratoria*[9] and *Choristoneura fumiferana*[10], 26-hydroxy-β-ecdysone has been suggested to occur in the radiochromatograms of the tissue extracts but no positive identifications were made. We have now identified 26-hydroxy-β-ecdysone as a metabolite of β-ecdysone in the blowfly, *Calliphora erythrocephala.*

Late 3rd-instar larvae (115) of *Calliphora erythrocephala* were injected with 5-[^{3}H]-β-ecdysone[8] (1.5 μg/insect, sp. act. 7.2 mCi/mM) and 20 h later, when they had all formed puparia, they were homogenized with methanol and centrifuged. The supernatant representing a 47% recovery of activity (2.76×10^6 dpm), was evaporated to dryness and partitioned between hexane:diethyl ether:methanol:water (8:5:3:1); the aqueous phase was concentrated to remove the methanol and extracted with n-butanol (X3). The n-butanol phase was evaporated to dryness and chromatographed on thin layer plates (Merck precoats, Silica gel HF_{254}) to give 3 radioactive peaks (figure). Peaks II and III co-chromatographed with 26-hydroxy-β-ecdysone[11] and β-ecdysone respectively. The material of peak II was eluted from the plate with methanol and accounted for 10% of the recovered activity (2.74×10^5 dpm). The aqueous phase from the n-butanol extraction accounted for 52% of the activity of the ethanol extract (1.43×10^6 dpm) and contained only ecdysone conjugates[12].

Since the 26-hydroxy-β-ecdysone standard was non-crystalline[13] the identity of the material from peak II was confirmed by the preparation of derivatives. A portion of the material was reacted with acetone (1 ml, 20 °C) in the presence of phosphomolybdic acid under nitrogen[14]. The acetonide mixture was examined by TLC ($CHCl_3$:EtOH, 19:1) and compared with 26-hydroxy-β-ecdysone reacted with acetone under identical conditions. The radiochromatogram showed a pattern of peaks whose R_f values were the same as the components in the 26-hydroxy-β-ecdysone acetonide mixture. The main peak co-chromatographed with the major component, 26-hydroxy-β-ecdysone triacetonide[13].

Acetylation[14] (24 h) of the material from peak II followed by TLC ($CHCl_3$:EtOH, 19:1) also gave a radiochromatogram in which the peaks co-incided with spots in the acetylation mixture of 26-hydroxy-β-ecdysone. The main peak co-chromatographed with the main acetylation product, the tetra-acetate[13].

The co-chromatography of the metabolite and its derivatives with 26-hydroxy-β-ecdysone and its derivatives prepared under identical conditions, confirms the identity of 26-hydroxy-β-ecdysone as a metabolite of β-ecdysone in *Calliphora erythrocephala.*



Thin-layer radiochromatogram of the n-butanol phase of the total ethanol extract of late 3rd-instar *Calliphora erythrocephala* larvae injected with β-5-[^{3}H]-ecdysone. *a* 26-Hydroxy-β-ecdysone, *b* β-ecdysone (vanillin/H_2SO_4 spray reagent). Solvent system: $CHCl_3$:EtOH (2:1, v/v). Peak I is at the origin.