On the components of the defensive substances of stink bugs

| GLC $t_R$ (2-hexenal = 1) | Assigned | *A. magna* composition, % | *M. japonensis* | *A. nigritus* | Method of identification |
|---|---|---|---|---|---|
| 0.94 | unknown | 2.7 | | | |
| 1.00 | 2-hexenal | 91.5 | 11.2 | | GLC, IR, NMR, 2,4-DNP |
| 1.95 | 2-octenal | 5.8 | 24.6 | 4.7 | GLC, IR |
| 2.10 | *n*-tridecane | | 61.8 | 2.0 | GLC, MS |
| 2.72 | 2-decenal | | 2.4 | 34.6 | GLC, IR, 2,4-DNP |
| 2.82 | *n*-pentadecane | | | 58.7 | GLC, MS |

*medius* Dist., *n*-dodecane, *n*-tridecane, *n*-pentadecane, hexanal, 2-hexenal, 4-oxo-2-hexen-1-al, 2-octen-1-al, 4-oxo-2-octen-1-al.

*Material and method.* We have investigated the defensive substances of 3 species of Cydnidae in Japan: *Adrisa magna* Uhler, *Macroscytus japonensis* Scott, *Aethus nigritus* Fabricius. The stink bugs were collected in the ground on April in Hiroshima Prefecture, Japan. The stink bugs were irritated and they then secreted the defensive substance in the test tube. After taking the bugs out of the test tube, the secretion was extracted with *n*-hexane. Evaporation of the solvent gave odorus principles. The ordorus substance of 1.2 mg was obtained from an irritated example of *A. magna*, 0.18 mg of secretion was obtained of *M. japonensis* and 0.08 mg of *A. nigritus*. The idividual components of the secretion were isolated by the preparative gas chromatography using SE30 (10%) column and identified by gas chromatography (GLC), IR-spectrum, NMR-spectrum or mass spectrum (MS) compared with those of authentic specimens. Some of alkenals were further identified by the preparation of 2,4-dinitrophenylphydrazone (2,4-DNP) with Brady's reagent (2,4-dinitrophenylphydrazine). *n*-Alkenals were synthesized from the corresponding *n*-alkanals according to the BEDOUKIAN's method[9]. For analysis Hitachi K53 gas chromatograph equipped with flame ionization detector was used. The gas chromatograph was operated with the temperature programmed from 50 to 160° (3 °C/min) using HB2000 capillary column (0.25 mm × 45 m). The relative percentages of the individual components shown in the Table were determined by integration and summation of the peak areas with electronic digital integrator. Retention time was also determined by the integrator. 2-Hexenal; $t_R$ 1.00 (10.38 min) IR ($CCl_4$) 2960, 2940, 2880, 2810, 2720, 1690, 1638, 1458, 1381, 1341, 1301, 1280, 1150, 1140, 1090, 1043, 1002, 970 $cm^{-1}$. NMR ($CCl_4$); $\delta$ 9.50 (1H), 6.86 (1H), 6.07 (1H), 2.30 (2H), 1.45 (2H), 0.93 (3H). 2,4-DNP m.p. 146.5 °C (mixed m.p. not depressed). 2-Octenal; $t_R$ 1.95 IR ($CCl_4$) 2970, 2945, 2865, 2810, 2725, 1700, 1642, 1475, 1460, 1440, 1385, 1304, 1158, 1144, 1102, 1048, 982 $cm^{-1}$. 2-Decenal; $t_R$ 2.72 IR ($CCl_4$) 2962, 2943, 2862, 2725, 1700, 1642, 1472, 1440, 1385, 1300, 1155, 1142, 1100, 978 $cm^{-1}$. 2,4-DNP m.p. 126.5 °C (mixed m.p. not depressed). *n*-Tridecane; $t_R$ 2.10, MS *m/e* 184 ($M^+$), 154, 140, 126, 112, 99, 85, 71, 57 (base peak), 43. *n*-Pentadecane $t_R$ 2.82 MS *m/e* 212 ($M^+$).

*Results and discussion.* The secretion of *A. magna* contained 2-hexenal (91.5%) and 2-octenal (5.8%) as the main components. The defensive substance of *M. japonensis* identified as 2-hexenal (11.2%), 2-octenal (24.6%) and *n*-undecane (61.8%). *n*-Pentadecane (58.7%) was found in the secretion of *A. nigritus* together with 2-octenal (4.7%), 2-decenal (34.6%) and *n*-tridecane (2.0%). *n*-Alkenal was widely spread through-out Pentatomidae, Coreidae, and Cimex families. The secretion of *M. japonensis* and *A. nigritus* contained the *n*-paraffins as the main components, while no *n*-paraffin was found in the secretion of *A. magna*. These results can be used for the chemosystematic study of the stink bugs. It can be seen that *n*-paraffins in the secretion were used as the solvent of the *n*-alkenals: in the case of *n*-paraffins as the solvent of formic acid in the ants[10].

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## NMR-Studies of Triiodothyropropionic Acid in Ethanol-HCl

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*Summary.* The barrier to rotation in the N-acetyl methyl ester of thyroxine was found to be 8.6 kcal $mol^{-1}$. Previous experiments determining the barrier to rotation in triiodothyropropionic acid in HCl-ethanol were shown to be in error.

The conformations of the active thyroid hormone 3,5,3′-triiodo-L-thyronine ($T_3$) have been extensively studied. Steric effects of the 3,5-iodines force the 2 aromatic rings to lie in mutually perpendicular planes (Figure 1), resulting in the formation of 2 conformational isomers which differ only in the orientation of the 3′-iodine with respect to the inner ($\alpha$) aromatic ring. When the 3′-iodine lies over the $\alpha$-ring the conformation is termed proximal, and it is distal when the 3′-iodine is away from the $\alpha$-ring. JORGENSEN et al.[3,4] have con-

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cluded from biological testing results on conformationally immobile systems that the distal arrangement is the important one for hormonal activity.

A number of X-ray crystallographic determinations on $T_3$ analogs have given varied results. Triiodothyropropionic acid ethyl ester ($T_3P$ ethyl ester)[5], $T_3$ hydrochloride[6] and 3'-isopropyl-3,5-diiodo-L-thyronine hydrochloride[7] were shown to exist in the proximal conformation in the crystalline state, whereas $T_3$[8], $T_3$ methyl ester[9] and 3,5,3'-triiodothyroacetic acid, N,N-diethanolamine[10] were shown to exist in the distal arrangement.

We have studied the low temperature NMR-spectrum of the N-acetyl methyl ester of thyroxine ($T_4$) in acetone $-D_6$. At room temperature the aromatic portion of the spectrum appears as 2 peaks at $\delta$ 7.28 (2' and 5' protons) and $\delta$ 7.96 (2 and 5 protons). At $-94\,°C$ the 2'- and 5'-protons appear at $\delta$ 6.64 and $\delta$ 7.92 respectively. Coalescence occurs at $-88\,°C$ giving a value of $\Delta G = 8.6 \pm 0.2$ kcal mol$^{-1}$ entirely consistent with barriers to rotation of 7.9 and 8.5 kcal mol$^{-1}$ recently determined for $T_3$ and $T_4$ model compounds[11].

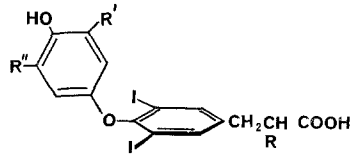


Fig. 1. Drawing showing thyroxine R = $NH_2$, R' = R'' = I and proximal (R = R'' = H, R' = I) and distal (R = R' = H, R'' = I) conformations of triiodothyropropionic acid ($T_3P$).

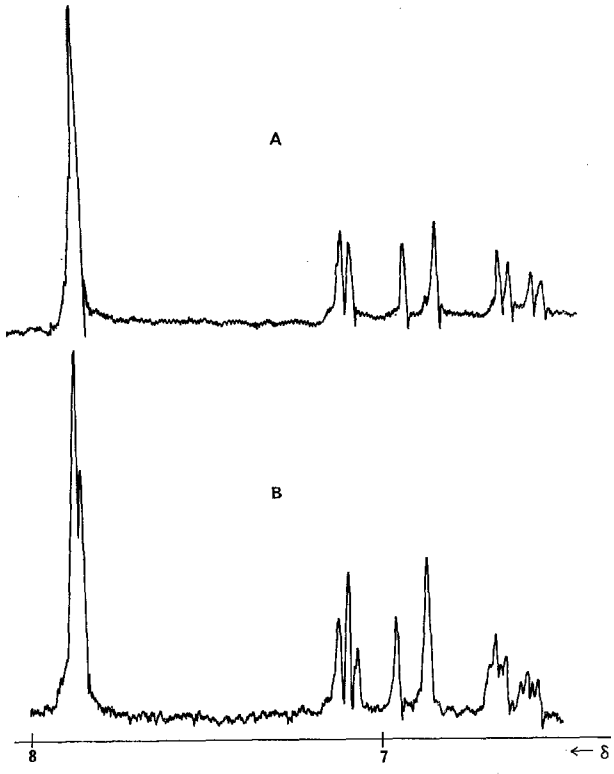


Fig. 2. A) 100 MHz Proton-NMR-spectrum of $T_3P$ in ethanol (250 µl) – water (16 µl) immediately after the addition of HCl (2 µl – 38%). B) Sample A after standing for 18 h at room temperature. The appearance of the spectra shown above differs from that reported[10] because they were obtained at different field strengths, e.g. the triplet is, in fact, a pair of overlapping 2'-proton doublets, one each from the acid and ester. These 100 MHz spectra are entirely consistent with those taken at 220 MHz.

Recently N. CAMERMAN et al.[12] reported an NMR-study of the solution conformations of $T_3P$. They reasoned that, since all of the materials which showed proximal arrangements in the solid state were crystallized from acidic (HCl) solutions, acidic media might be affecting the solution conformation and in some way causing a preponderance of the proximal conformer. Accordingly they obtained a spectrum of a sample of $T_3P$ dissolved in acidified (HCl) aqueous ethanol. The spectrum clearly showed that 2 species were present in an approximately 3:1 ratio and they interpreted the spectral data on the basis of a mixture of proximal (major) and distal conformations of $T_3P$ in solution. They also noted that heating the sample to 60° caused no coalescence of peaks indicating a rotational barrier in excess of 20 kcal mol$^{-1}$. CAMERMAN et al.[12] could offer no explanation for the extreme effect of acidification or the large barrier to rotation. Their results were so surprizing, and important if correct, that we felt a reinvestigation was warranted.

Our initial attempts to reproduce the reported spectrum at 220 MHz were unsuccessful. We were unable to reproduce the reported[12] solubility of $T_3P$ in 2:1 ethanol-1 *N* HCl, and the resonance for the 5'-proton doublet of $T_3P$ in 2:1 ethanol-$H_2O$ was displaced 0.045 ppm downfield from that in the reported spectrum. However, the spectrum could be reproduced in 7% aqueous ethanol solvent.

We have carried out the following experiments: 1. A spectrum of $T_3P$ in 7% aqueous ethanol was determined and shown to reproduce the spectrum reported for the 'minor species'. 2. A spectrum of $T_3P$ ethyl ester in aqueous ethanol reproduced that reported for the 'major species'. 3. A mixture of $T_3P$ and $T_3P$-ethyl ester in aqueous ethanol reproduced the spectrum reported[12] for '$T_3P$' in ethanol-HCl. 4. A spectrum of $T_3P$ in aqueous ethanol was recorded, concentrated HCl added, and the spectrum retaken after 15 min (Figure 2A). The spectrum obtained was clearly of a single species ($T_3P$). On standing overnight (18 h) at room temperature, the sample gave a spectrum identical with the $T_3P$-$T_3P$-ethyl ester mixture spectrum (Figure 2B).

The obvious conclusion is that the 2 species present are $T_3P$ and its ethyl ester formed from $T_3P$ in the ethanol-HCl mixture (Fisher esterfication). No coalescence of peaks occur on heating because the 2 species present are different compounds and not different conformations.

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