TABLE II

Antifungal and antibacterial activities M. I. C. (μ g/ml)

(*a*)

Compound No.	*Fungi*									
	T.m.	*T.r.*	*M.c.*	*M.g.*	*C.a.*	*C.n.*	*S.s.*	*H.c.*	*A.f.*	*A.t.*
1	..	..	..	100	..	..	100	25	..	..
2	100	..	..	..	..	..	100	100	..	..
3	100	100	..	100	..	..	100	100	..	100
4	50	..	100	100	..	..	100	25	..	..
5	50	50	25	25	..	..	50	25	..	..
6	50	50	50	50	100	100	50	25	..	100

(*b*)

	Bacteria						
	S.a.	*S.f.*	*E.c.*	*K.p.*	*Ps.a.*	*S.t.*	*Ag.t.*
5	50	25	..	50	25	..	50
6	..	..	..	..	100	..	..
11	..	..	..	100	25	..	25
13	..	..	..	..	..	..	50

Fungi: *T.m.* = *Trichophyton mentagrophytes*; *T.r.* = *Trichophyton rubrum*; *M.c.* = *Microsporum canis*; *M.g.* = *Microsporum gypseum*; *C.a.* = *Candida albicans*; *C.n.* = *Cryptococcus neoformans*; *S.s.* = *Sporotrichum schenkii*; *H.c.* = *Histoplasma capsulatum*; *A.f.* = *Aspergillus fumigatus*; *A.t.* = *Alternaria tenuis*.

Bacteria: *S.a.* = *Staphylococcus aureus*; *S.f.* = *Streptococcus faecalis*; *E.c.* = *Escherichia coli*; *K.p.* = *Klebsiella pneumoniae*; *Ps.a.* = *Pseudomonas aeruginosa*; *S.t.* = *Salmonella typhi*; *Ag.t.* = *Agrobacterium tumefaciens*.

.. = *Inactive*

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1. Mirua, K., *Progress in Medicinal Chemistry*, Edited by Ellis, G. P. and West, G. B., Published by Butterworths, London, 1967, 5, 320.
2. Noguchi, T., Hashimoto, Y., Kosaka, S., Kikuchi, M., Miyazaki, K., Sakimoto, R. and Kaji, A., *Yakugaku Zasshi*, 1968, 88 (3), 344 ; *Chem. Abstr.*, 1968, 69, 93987 *q*.
3. Actor, P., Anderson, E. L., Dicuollo, C. J., Ferlanto, R. J., Hoover, J. R. E., Pagano, J. F., Ravin, L. R., Schiedy, S. F., Stedman, R. J. and Theodorides, V. J., *Nature*, 1967, 215, 321.
4. Amorosa, M. and Liparini, L., *Ann. Chem.*, 1956, 46, 343.
5. Saikachi, H. and Takai, K., *Yakugaku Zasshi*, 1968, 88 (9), 1189 ; *Chem. Abstr.*, 1969, 70, 47189d.
6. Baker, J. W., Schumacher, I. and Roman, D. P., *Medicinal Chemistry*, Part I, 3rd edition, edited by Alfred Burger, published by Wiley-Interscience, New York, 1969, p. 636.
7. Singleton, H. M. and Edwards, W. R. Jr., *J. Am. Chem. Soc.*, 1938, 60, 541.
8. Robinson, R. C. V., Ferciot, T. N. III and Robinson, H. M. Jr., *AMA. Arch. Der.*, 1960, 81, 681.
9. Sutherland, R., Elson, S. and Croydon, E. A. P., *Chemotherapy*, 1972, 17, 145.

INFLUENCE OF SOLVENTS ON THE CHELATION IN BENZOIN AND METHYL MANDELATE

INTRAMOLECULAR hydrogen bonding in six membered chelates as formed in salicylaldehyde, methyl salicylate, 2-hydroxy acetophenone and enols of β-diketones has been studied earlier[1]. The intramolecular hydrogen bonding leading to the formation of five membered chelate, however, received little attention.

In this communication, evidence for chelation in benzoin and methyl mandelate is furnished. With a view to studying the influence of solvents on chelation the pmr spectra of these compounds have been recorded on Varian A. 60 D in CS_2, $CDCl_3$, DMSO, DMF, acetone and in the presence of traces of trifluoroacetic acid and methanol using TMS as internal standard at 37° C. The

concentrations of the solutions used are of the order of 0·1 molar. The results are presented in Table I.

TABLE I

CH and OH chemical shifts of benzoin and methyl mandelate in different solvents

Solvent	Benzoin		Methyl mandelate	
	CH δ (ppm)	OH δ (ppm)	CH δ (ppm)	OH δ (ppm)
CS_2	5·73	4·10	4·90	3·22
	5·83	4·20	5·00	3·32
$CDCl_3$	5·90	4·52	5·20	3·30
	6·00	4·62	5·30	3·40
,, $+D_2O$	5·90	..	5·20	..
,, +TFA	6·10	..	5·58	..
,, $+CH_3OH$	6·00	..	5·17	..
Acetone	6·10	4·85	5·20	4·67
	6·20	4·95	5·30	4·77
DMF	6·15	5·85	5·23	5·87
	6·25	5·95	5·36	5·97
DMSO	5·90	6·05	5·13	5·95
	6·00	6·15	5·22	6·05

TFA = Trifluoro acetic acid
DMF = Dimethylformamide
DMSO = Dimethylsulphoxide

The spectra of benzoin and methyl mandelate were reported earlier[2,3] but no study has been made in regard to chelation in these compounds. Both the compounds are found to exhibit doublets ($J = 6·0$ Hz) for proton signals of OH and CH of CHOH group. This is in contrast to the earlier report[2] where singlets were reported for OH and CH protons. The splitting of OH and CH signals may be due to coupling between them. In presence of D_2O, trifluoroacetic acid and methanol, the OH signals vanished and the CH signals collapsed into singlets. Such a collapse of the fine structure of the proton signals due to chemical exchange was reported earlier in the case of methanol[4]. In the presence of acids or bases the CH_3 doublet of methanol collapses into a singlet due to rapid OH proton exchange. The CH_3 signal, however, appeared as a doublet in the presence of sufficient amount of acetone.

On hydrogen bond formation between the C=O (acetone) and –OH (methanol) the resident time of the OH proton in the hydroxyl group increases when coupling with methyl group becomes possible. The doublet signals for CH of –CHOH in benzoin and methyl mandelate therefore suggest bonding between OH and >C=O, leading to the formation of a five membered chelate. This is also reflected in the downfield shift of OH proton in $CDCl_3$ of benzoin (4·57 δ) and of methyl mandelate (3·35 δ), as compared to the OH proton in benzyl alcohol (2·43 δ)[5].

The relative insensitivity of the OH proton signals to the changes in concentration further support the formation of intramolecular hydrogen bonding. The downfield shift of the OH proton in these compounds is not so great as in other chelate compounds, *viz.*, salicylaldehyde, 2-hydroxy acetophenone and enols of β-diketones. Chelation in these compounds, therefore, appears to be weak. The weak chelation may be due to the absence of conjugation in the chelate ring, as found in the compounds mentioned above. The larger downfield shift of OH proton in benzoin as compared to that in methyl mandelate may be due to greater electron donating ability of the oxygen of the ketonic >C=O than that of the ester >C=O. The lone pair electrons on the oxygen of the ketonic >C=O approach the OH proton more closely and repel greatly the electron which is in the nieghbourhood of the hydrogen nucleus and therefore reduce the diamagnetic shielding of that nucleus by its own electrons and increase the paramagnetic deshielding[6].

The influence of solvents on the chemical shifts of CH and OH protons of benzoin and methyl mandelate is rather interesting. The signals of both the protons remain as doublets indicating the absence of OH proton exchange as noticed in the presence of D_2O, trifluoro acetic acid and methanol. With the increase in polarity of the solvent from CS_2 to DMSO the OH signals in general move to lower fields, the shifts being larger in *n*-donor solvents like DMSO, DMF and acetone.

Although the OH chemical shifts of benzoin and methyl mandelate are different in $CDCl_3$ and CS_2 both of them have almost the same value either in DMF and DMSO. This may happen when the OH groups of the two compounds are bonded to the same donor. In DMSO and DMF, the chelation may be ruptured and intermolecular hydrogen bonds may be formed with more basic solvent molecules as shown in Fig. 1.

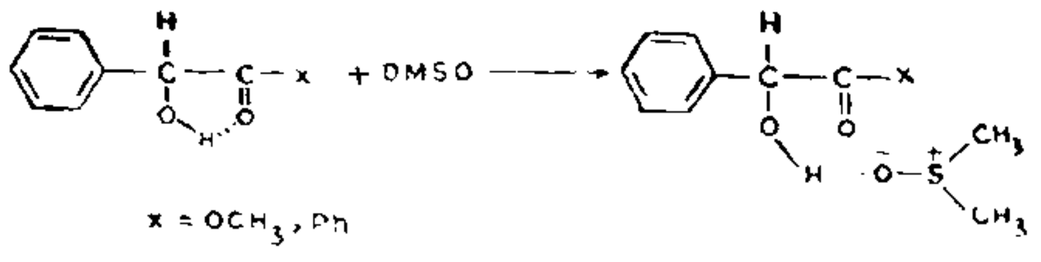

FIG. 1

This view is in agreement with an earlier finding[7] that the chelation in salicylaldehyde is disrupted with DMSO leading to the formation of an intermolecular hydrogen bonding.

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April 25, 1974. N. V. SUBBA RAO.

1. Hay, R. W. and Williams, P. P., *J. Chem. Soc.*, 1964, p. 2270.
2. Okumura, Y., *Repts. of Fac. of Sci., Shizuoka Univ.*, 1970, 5, 41.
3. Mori, N., Tanaka, Y. and Tsuzuki, Y., *Bull. Chem. Soc., Japan*, 1966, 39, 1490.
4. Corio, P. L., Rutledge, R. L. and Zimmerman, J. R., *J. Am. Chem. Soc.*, 1958, 80, 3163.
5. *NMR Spectra Catalogue,* Varian Associates, Spectrum No. 161, 1964.
6. Porte, A. L., Gutowsky, H. S. and Hunsberger, I. M., *J. Am. Chem. Soc.*, 1960, 82, 5057.
7. Karabatsos, J. and Vane, F. M., *Ibid.*, 1963, 85, 3886.

ECOPHYSIOLOGY OF A HOST-PARASITE SYSTEM : EFFECT OF INFECTION OF A PARASITIC COPEPOD, *LERNAEA HESARGATTENSIS* ON THE OXYGEN CONSUMPTION OF THE FISH, *LEBISTES RETICULATUS* PETERS)

WORK on the physiology of copepods, especially of the parasitic forms, is wanting[1]. A new species of a parasitic copepod *Lernaea hesargattensis,* infecting the cyprinodont fish *Lebistes reticulatus,* has been recently described[2,3]. The adult female parasites are found to be firmly embedded in the muscular tissue of the host and thus obtain their nutritional requirement.

One of the vital ecological factors affecting the survival of fish is the availability of oxygen in the aquatic habitats and the oxygen consumption of the fish is a direct index to its metabolic rate and food requirement[4]. Hence, in the present paper, the effects of infection of *L. hesargattensis* on the oxygen consumption of the fish *L. reticulatus* is described.

The test fish were collected from the fish ponds of Hesarghatta, near Bangalore. The fish were separated into the following experimental series : (1) Normal, (2) infected, with the parasite intact and (3) infected, with the parasites removed. Oxygen consumption of all these fish were estimated for males and females separately, using the modified Winkler's method[5]. In many of the infected fish, at the region of penetration of the parasite, certain tissue damage and inflammation around the area was observed. This reaction was local and did not extend beyond the area of infection. Similar local reactions were recorded in salmonids due to infection by the leach *Piscicola salmositica*[6].

Table I represents the average values of oxygen consumed by males and females of the experimental series. A normal male fish consumed $0{\cdot}2685 \pm 0{\cdot}051$ cc of oxygen/gram body weight/hour. During the same period the infected fish consumed as much as $0{\cdot}3314 \pm 0{\cdot}139$ cc of oxygen/gram body weight.

TABLE I

Effect of infection of the copepod parasite Lernaea hesargattensis *on the oxygen consumption of male and female* Lebistes reticulatus. *Each value represents the mean of 6 experiments*

Material	Sex	Body weight (mg)	Oxygen consumed/ g body weight/hour (cc)
Normal	Male	196·80 ± 13·83	0·2685 ±0·051
Controls	Female	264·27 ± 22·67	0·7725 ±0·320
Infected, with parasite	Male	174·87 ± 12·78	0·3314 ±0·139
	Female	329·05 ± 26·92	0·8981 ±0·084
Infected, without parasite	Male	114·58 ± 8·94	0·2608 ±0·159
	Female	330·20 ± 31·22	0·6614 ±0·332

The corresponding value for normal and infected females were $0{\cdot}7725 \pm 0{\cdot}320$ and $0{\cdot}8981 \pm 0{\cdot}084$ cc of oxygen/gram body weight/hour, respectively. The higher consumption by female *Lebistes,* despite a larger body weight when compared to males, may be due to the fact that these experiments were conducted during March 1973 when the laboratory water temperature was 32° C, five degrees more than the temperature at which the males were experimented (November, 1972).

In both male and female *Lebistes,* when the parasites were removed, the oxygen consumption dropped back almost to the normal values. In the males, on removal of the parasite, the fish consumed $0{\cdot}2608 \pm 0{\cdot}159$ cc of oxygen/gram body weight/hour and in the females, the corresponding value was $0{\cdot}6614 \pm 0{\cdot}332$ cc. Moreover, the oxygen consumed by the parasite alone (after it was removed from the host's tissue) was found to be negligible. Hence, this increased oxygen consumption of the infected *Lebistes,* irrespective of the sex of the fish, can be attributed to a 'stress reaction' due to parasitic infection of the copepod.

The precentage increase in the oxygen consumed, from the normal to infected males, was 20·42 while in females this increase was slightly less (16·30%).