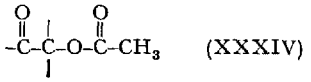
hand, the corresponding six-membered ring analog (XXXII) and the acyclic analog ethyl levulinate (XXXIII) show carbonyl bands at normal positions.

It therefore appears that the interaction between the carbonyl groups both in 1,4-diketones and in 1,4-keto-esters occurs only when one ketone is in a five-membered ring, presumably because the latter renders the system more rigid.

The presently described carbonyl-carbonyl interactions in 1,4-dicarbonyl compounds are probably of the same type as exist in α-acetoxy-ketones of type (XXXIV), which may also be considered as 1,4-dicarbonyl systems. Thus, in 21-acetoxy-20-keto-steroids the ketone band as well as the ester band is raised by ca. 20 cm^{-1} over the normal values[5,7,8] while in 17α- and 17β-acetoxy-20-keto-steroids the ketone band is raised by ca. 8 cm^{-1} (the acetate band being essentially unaffected)[8,9]. Similar effects have been found in steroidal 12-acetoxy-11-ones and 11-acetoxy-12-ones[10].



(XXXIV)

It has already been postulated by JONES *et al.*[8,11] that the anomalous infrared spectra of these α-acetoxy-ketones are due to the existence of a field effect between the two carbonyl groups and BELLAMY and WILLIAMS[12] have provided evidence for this by pointing out that the anomaly is markedly dependant on steric factors. The steric nature of this effect is also shown well by our finding (Table V) that whereas the expected raising of the infrared carbonyl bands occurs with acetol acetate (XXXVI) (+ 21 cm^{-1} for the ketone band; + 21 cm^{-1} for the ester band) it is considerably less marked in acetol pivalate (+ 11 cm^{-1} and 18 cm^{-1}, respectively) in which the extra methyl groups prevent the carbonyl dipoles from being as close to each other as in the acetate.

Table V

Compound	$\nu_{max}(CHCl_3)$[a]
CH_3COCH_3 (XXI)	1712 cm^{-1}
$CH_3COOC_2H_5$ (XXIX)	1726 cm^{-1}
$(CH_3)_3COOCH_3$ (XXXV)	1718 cm^{-1}
$CH_3COCH_2OCOCH_3$ (XXXVI)[b] . .	1733, 1747 cm^{-1}
$CH_3COCH_2OCOC(CH_3)_3$ (XXXVII)[b]	1723, 1736 cm^{-1}

[a] All values determined by ourselves.

[b] Prepared in these Laboratories by reaction of α-chloro-acetone with the corresponding potassium alkoxide.

All the infrared data discussed clearly point to the existence of an intramolecular field effect between the carbonyl groups in certain 1,4-dicarbonyl compounds. Although the exact nature of this effect is still not well understood, it must operate between the dipoles across space and is clearly different in nature from inductive and mesomeric effects which are transmitted through the chain.

We thank Dr. S. PINCHAS, Weizmann Institute of Science, who determined most of the infrared spectra on a Perkin-Elmer model 12 C single-beam spectrophotometer (sodium chloride optics).

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Daniel Sieff Research Institute, Weizmann Institute of Science, Rehovoth (Israel), January 5, 1960.

Zusammenfassung

Es wird gezeigt, dass die IR-Spektren gewisser 1,4-Diketone und 1,4-Ketoester abnorm hohe Carbonylfrequenzen aufweisen, was auf das Vorhandensein eines intramolekularen Feldeffekts zwischen den beiden Carbonylgruppen in diesen Verbindungen hindeutet. Die ungewöhnlichen Stabilitätsverhältnisse an C-20 der 16,22-Dicarbonyl-Steroide sind vermutlich auf die gleiche 1,4-Carbonyl-Carbonyl-Einwirkung zurückzuführen.

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[7] R. N. JONES, P. HUMPHRIES, and K. DOBRINER, J. Amer. chem. Soc. *72*, 956 (1950).

[8] R. N. JONES, P. HUMPHRIES, F. HERLING, and K. DOBRINER, J. Amer. chem Soc. *74*, 2820 (1952).

[9] R. B. TURNER, J. Amer. chem. Soc. *75*, 3489 (1953).

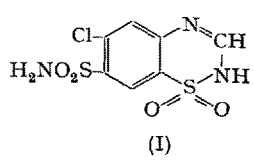
[10] J. ELKS, G. H. PHILLIPS, T. WALKER, and L. J. WYMAN, J. chem. Soc. *1956*, 4330.

[11] R. N. JONES and C. SANDORFY, *Chemical Applications of Spectroscopy* (Interscience, New York 1956).

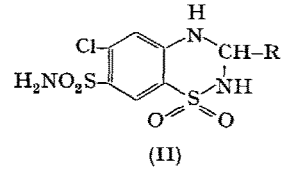
[12] L. J. BELLAMY and R. L. WILLIAMS, J. chem. Soc. *1957*, 861.

3-Haloalkyl-Dihydrobenzothiadiazine Dioxides as Potent Diuretic Agents

The class of compounds based on the 1,2,4-benzothiadiazine nucleus became important from the standpoint of pharmacological activity with the discovery that 6-chloro-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide (I) was a potent, orally effective diuretic agent of low toxicity[1].



(I)



(II)

As part of a program directed towards structural modifications of I, with the view of finding other agents of increased activity along with superior electrolyte excretion patterns, we have prepared a series of compounds

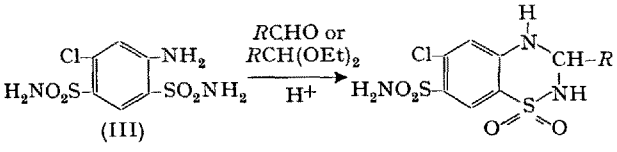
[1] F. C. NOVELLO and J. M. SPRAGUE, J. Amer. chem. Soc. *79*, 2028 (1957). The generic name of this compound is chlorothiazide.

[2] G. DE STEVENS, L. H. WERNER, A. HALAMANDARIS, and S. RICCA JR., Exper. *14*, 463 (1958). The generic name of this compound is hydrochlorothiazide.

[3] Hahnemann Medical College and Hospital, *Symposium on Edema*, Philadelphia, Pa., December 7 to 11 (1959).

represented by formula II. While this work was in progress, DE STEVENS *et al.* described the synthesis and diuretic activity of 6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide, II (R = H)[2]. From our own findings and those reported by DE STEVENS *et al.*, it was apparent that hydrogenation of the 3,4 double bond in I resulted in a compound with at least a ten fold increase in potency together with a somewhat improved electrolyte excretion pattern. With variation in R this relationship seemed to hold for most cases.

Compounds of type II were prepared by condensation of 5-chloro-2,4-disulfamylaniline (III) with the appropriate aldehyde or its acetal in a suitable solvent, usually in the presence of an acid catalyst.



Of the many types of 3-substituents in the 3-substituted 3,4-dihydrobenzothiadiazine dioxides thus synthesized the 3-haloalkyl series was among the more noteworthy. In the halomethyl series the dihalomethyl compounds were found to be far more active than their mono or trihalomethyl counterparts as determined by oral studies in animals. Thus the activities of the monochloromethyl, dichloromethyl, and trichloromethyl compounds were found to be approximately two, fifteen, and one-half, respectively, relative to hydrochlorothiazide taken as one.

3-Dichloromethyl-6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide, II, $R = CHCl_2$, has been extensively tested orally in rats and dogs for its effect on electrolyte excretion and urine flow rates. These studies showed that the compound was ten to twenty times as potent as hydrochlorothiazide, one hundred to two hundred times as potent as chlorothiazide and exhibited a low order of toxicity. The greatly increased sodium and chloride ion excretion rates showed virtual correspondence with the enhanced urine flow rate. The output of potassium and bicarbonate ions was not materially affected and in this connection it is interesting that the compound was found to be a very weak carbonic anhydrase inhibitor. Clinical trials[3] have confirmed the potency ratio determined in animal experiments and have also demonstrated a significant reduction in the excretion of potassium ion relative to sodium ion compared with chlorothiazide and hydrochlorothiazide.

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N. SPERBER, and J. TOPLISS

Medicinal Chemical Research Department, Schering Corporation, Bloomfield (New Jersey), January 12, 1960.

Zusammenfassung

Die Synthese und diuretische Aktivität von 3-Haloalkyl-dihydrobenzothiadiazin-dioxyden, insbesondere 3-Dichloromethyl-6-chlor-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazin-1,1-dioxyd, II, $R = CHCl_2$, werden beschrieben.

Some Histochemical Observations on the Cuticle of Fasciola indica Verma, 1953

Presence of -S-S- bonding and polyphenol which may act as precursor of tanning, have been reported in the external cortical layer of certain nematodes like *Ascaris* and *Proleptus obtuses*[1–3]; but little attention seems to have been paid to the study of cuticle of trematodes. In the present investigation the authors have made histochemical observations on the cuticle of the trematode *Fasciola indica* Verma, 1953, recovered from the liver of freshly killed buffalo. Live specimens of the trematode brought to the laboratory in a thermos-flask containing physiological saline at 37°C were used for this purpose. Frozen and paraffin sections, as well as thin strips cut along the margin of the flattened worms (containing only the integument), were studied. Methods described by COWDRY[4], PEARSE[5], TRIM[6], and SMYTH[7,8] were employed for testing presence of proteins, fats, phenols, aromatic crosslinkage, and -S-S- bonds.

The results of the various histochemical tests on the cuticle of *Fasciola indica* have been summarized in the following Table.

Groups	Tests	Results
Proteins	Biuret	Positive
	Xanthoproteic	Positive
Glycol group	P.A.S.	Positive (pink)
	P.A.S. after chloroform extraction	Positive
	P.A.S. after saliva treatment	Positive
	P.A.S. after acetylation	Negative
	P.A.S. after 0·1 *N* KOH treatment of acetylized sections	Positive
	γ-metachromasia with Toluidine blue	Negative
	Methylene blue binding below pH 4	Negative
Phenols, aromatic linkage	Ferric chloride	Negative
	0·2% Catechol	Negative
	Malachite green (G. T. GURR)	Negative
-S-S- bonds	Thioglyocollate	Negative
	Alkaline lead acetate	Negative
Fats	Sudan IV	Negative

The positive Biuret and Xanthoproteic tests obtained in this work are sufficient evidences of the proteinaceous nature of the cuticle. The cuticle also gave a positive P. A. S. reaction. However, the nature of the exact substance causing this reaction could be ascertained only after chloroform extraction, saliva treatment, acetylation, and lastly after alkali treatment of acetylized material, and this in logical sequence demonstrated the presence of

[1] C. H. BROWN, Nature *165*, 275 (1950).
[2] C. H. BROWN, Personal communication to von Brand (1952).
[3] A. F. BIRD, *Exp. Parasitol. 6*, 383 (1957).
[4] E. V. COWDRY, *Laboratory technique in Biology and Medicine*, 2nd Ed. (The William & Wilkins Co., Baltimore 1948).
[5] A. G. E. PEARSE, *Histochemistry Theoretical & Applied* (J. & A. Churchill Ltd., London 1953).
[6] A. R. TRIM, Biochem. J. *35*, 1088 (1941).
[7] J. D. SMYTH, Stain techn. *26*, 255 (1951).
[8] J. D. SMYTH, Quart. J. micr. Sci. *95*, 139 (1954).