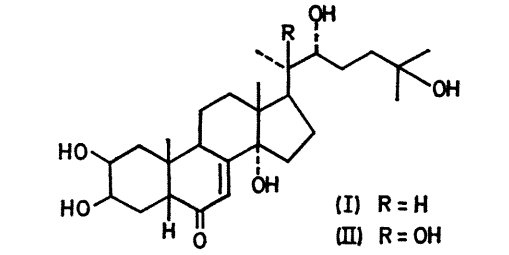
Insect Moulting Hormones (Ecdysones). Identification as Derivatives by Gas Chromatography

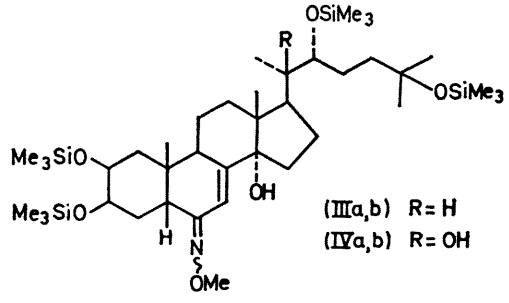
By E. D. MORGAN* and A. P. WOODBRIDGE
(*Department of Chemistry, Keele University, Keele, Staffs.* ST5 5BG)

Summary Derivatives suitable for the identification and quantitative determination by g.l.c. of the insect moulting hormones ecdysone and crustecdysone (ecdysterone) in biological materials have been prepared.

METHODS for the g.l.c. identification of polar steroids have been reported;[1,2] application to compounds of the ecdysone type (I, II), active as insect moulting hormones, would be very useful for detecting small amounts of these compounds. We have investigated conditions necessary to give high yields of derivatives of these hormones, that are sufficiently volatile for gas chromatography, and that can be used on crude mixtures for their determination.



(I) R = H
(II) R = OH



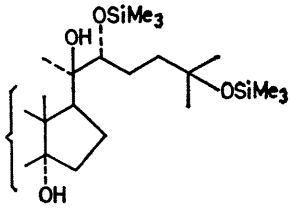
(IIIa,b) R = H
(IVa,b) R = OH

A solution of ecdysone (I) or crustecdysone (II) (2 mg), in purified pyridine (0·5 ml) was treated with *O*-methylhydroxylamine hydrochloride (8 mg) for 100 h at room temperature. In both cases the compound was converted almost quantitatively into two isomeric *O*-methyloximes, separable by preparative t.l.c. on silica gel with chloroform–95%-ethanol (80 : 20), developing three times. Each isomer was treated with excess of bis(trimethylsilyl)-acetamide (BSA) (0·5 ml) at room temperature for 70 h to yield silylated derivatives of the two hormones (IIIa and b) and (IVa and b). The R_F values on t.l.c., and retention times on g.l.c. using 5 ft silylated glass columns at 232°, with a nitrogen flow rate at 50 ml/min, are given in the Table. For identification and estimation, the separation of

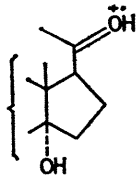
	R_F		G.l.c. retention times (min)	
	Chloroform–95%-ethanol (80 : 20)	Light petroleum-(60–80°)–ether (80 : 20)	3% QF-1 on CQ	1% OV-17 on CQ
(IIIa)	0·82	0·21	22·0	82·5
(IIIb)	0·82	0·32	24·5	138·5
(IVa)	0·82	0·10	31·5	121·5
(IVb)	0·82	0·21	33·0	195·5

isomers was not necessary and on the 3% QF-1 column the isomer mixtures gave characteristic double peaks.

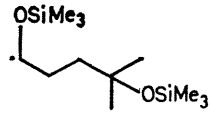
Mass spectrometry of the derivatives showed that they were pairs of isomeric *O*-methyloximes, as expected from a study of derivatives of the model compound 3β-hydroxy-5α-ergosta-7 : 22-dien-6-one,[3] and were in the incompletely silylated forms (IIIa, IIIb) (M^+ 781) and (IVa, IVb) (M^+ 797). Scission at C_{20}–C_{22}, giving rise to a prominent ion (V), *m/e* 536, with loss of the fully silylated fragment (VI), was observed in (IVa) and (IVb) followed by loss of 18 mass units (H_2O), and could be compared with the similar pattern in the fragmentation of crustecdysone.[4] More vigorous reaction conditions are necessary to silylate the tertiary 14α-hydroxy-group.[5]



(IVa,b)



(V)



(VI)

Treatment of partially purified extracts of 5th instar nymphs of the desert locust *Schistocerca gregaria*, with *O*-methylhydroxylamine hydrochloride and BSA, followed

by two dimensional t.l.c. in the solvent systems described, gave a derivative mixture identical in g.l.c. and m.s. with that obtained from authentic crustecdysone. Ecdysone was not detected, and if present must represent less than 1% of the amount of crustecdysone. Thus, by this method, groups of 10 nymphs of *S. gregaria* taken at different ages in the 5th instar were shown, after preliminary solvent partition, to have crustecdysone titers varying from 12 ng to 240 ng per individual.

We thank Dr. D. H. S. Horn of C.S.I.R.O., Melbourne for the gift of a sample of crustecdysone, and Professor P. Karlson, University of Marburg–Lahn for a sample of ecdysone. We acknowledge the award of a fellowship from the Anti-Locust Research Centre, London to A.P.W., and a grant from the S.R.C. for the purchase of g.l.c. equipment.

(*Received, March 3rd*, 1971; *Com.* 174.)

[1] M. G. Horning, A. M. Moss, and E. C. Horning, *Analyt. Biochem.*, 1968, **22**, 284.
[2] E. M. Chambaz and E. C. Horning, *Analyt. Letters*, 1967, **1**(3), 201.
[3] A. Furlenmeier, A. Furst, A. Langermann, G. Waldvogel, P. Hocks, U. Kerb, and P. Wiechert, *Experientia*, 1966, **22**, 573.
[4] M. N. Galbraith and D. H. S. Horn, *Austral. J. Chem.*, 1969, **22**, 1045.
[5] M. N. Galbraith, D. H. S. Horn, and E. J. Middleton, *Chem. Comm.*, 1968, 466.