Chemico-physical properties of the intermediates and of TRH

Compound (formula)	PF (°C)	$[\alpha]_D^{20}$	TLC[a]			Analysis (calc./found)		
			Rf¹	Rf²	Rf³	C	H	N
His–Pro–NH$_2$ · 2HBr (IV) | Bzl ($C_{18}H_{25}O_2N_5Br_2$)	196°–198°	−28.2° (c 1.0 in MeOH)	0.41	0.50	0.61	42.96 43.04	5.01 4.98	13.92 13.72
pGlu–His–Pro–NH$_2$ (VI) | Bzl ($C_{23}H_{28}O_4N_6$)	120°–121° (decomp.)	−24.5° (c 1.0 in MeOH)	0.48	0.54	0.76	61.05 61.35	6.24 6.12	18.57 18.50
pGlu–His–Pro–NH$_2$[b] (TRH) ($C_{16}H_{22}O_4N_6$–0.5 H_2O)	amorph.	−40° (c 1.0 in MeOH)	0.24	0.60	0.39	51.74 51.54	6.24 6.18	22.63 22.81

[a] The Rf¹, Rf² and Rf³ values (Silica Gel G according to Stahl activated to 110 °C for 1 h) refer to the eluent systems '*n*-butanol-glacial acetic acid-ethyl acetate-H_2O' (1:1:1:1); 'chloroform-methanol-NH_3 concentrated' (60:45:20); 'chloroform-methanol-acetic acid 30%' (60:45:20).
[b] The amino-acid analysis gave the following results: Glu 1.00; His 0.98; Pro 0.99; NH_3 0.96. The peptide hydrolysis was carried out in a closed ampoule in HCl 6 *N* at 110 °C for 48 h.

The homogeneity and the high grade of purity of the TRH obtained by the method reported in this paper were shown by thin layer chromatography and analysis of the amino acids and confirmed by its high biological activity. The valuation of the biodynamic activity was carried out on isolated rat pituitaries according to the method of SCHALLY et al.[13] which determines by radioimmunoassay the amount of TSH released by the pituitary after stimulation with TRH.

Summary. This synthesis of thyrotropin releasing hormone (TRH) is particularly suitable for large scale production of highly purified TRH, thanks to its high yields of the coupling and deprotection reactions together with the very simple final purification step.

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[14] The authors are indebted to Mr. I. LALLE for his excellent technical assistance.

Systemic Fungicidal Activity of *N*-[2,2,2-Trichloro-1-(3-pyridylamino)ethyl]-formamide

Triforine (I), *N,N'*-[piperazine-1,4-diyl-*bis*(2,2,2-trichloroethylidene)]-*bis*formamide[1–4], has attracted considerable interest as a systemic fungicide in plants particularly against powdery mildew and rust diseases. Several related *bis*formamides have been patented as fungicides[5]. We have recently been interested[3,6–9] in determining the salient chemical features necessary for systemic activity in compounds of this type. From these studies it emerged that compounds of structure (II) where R is O-alkyl, NH-alkyl or S-alkyl[7,8] were systemically active following root application against powdery mildews. The best compounds were fully inhibitory at 100 ppm. In contrast compounds (II) where R is O-aryl, NH-aryl or S-aryl were much less effective[7–9]. It has also been found that the 3 chlorine groups in (II; R=O-alkyl) can be replaced by bromine without significant loss of systemic activity although the hexabromo analogue of triforine was inactive[9].

We have now studied several compounds of type (II) were R is a pyridylamino or related heterocyclic moiety. One of these compounds, *N*-[2,2,2-trichloro-1-(3-pyridylamino)-ethyl]formamide (III) has been found to be outstanding as a systemic fungicide against *Erysiphe graminis* following root application to sand-grown wheat seedlings.

The compounds (Table I) were prepared by the following typical method (cf.[3]). To 1-(2,2,2-trichloro-1-formamidoethyl)pyridinium chloride[10] (0.005 mol.) suspended in

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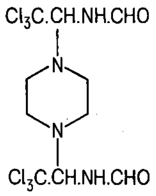
Table I. Chemicals

No.	Compound Structure II R	M.P.	Solvent	Formula	Analyses Found C	H	N	Required C	H	N
III	3-Pyridylamino	173	Benzene/petrol[a]	$C_8H_8Cl_3N_3O$	36.0	3.1	15.9	35.75	3.0	15.6
IV	2-Pyridylamino	190	Benzene	$C_8H_8Cl_3N_3O$	36.0	3.0	15.9	35.75	3.0	15.6
V	3-Quinolylamino	227 (dec.)	Benzene/chloroform	$C_{12}H_{10}Cl_3N_3O$	45.1	3.1	13.2	45.2	3.1	13.2
VI	2-Pyrimidylamino	174	Benzene/petrol[a]	$C_7H_7Cl_3N_4O$	30.9	2.8	20.6	31.2	2.6	20.8

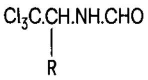
[a] Petrol as solvent refers to fraction of b.p. 60–80°.

acetone there was added the appropriate aminopyridine (0.01 mol.) dissolved in acetone. The mixture was refluxed for 2–8 h and was then filtered. The solvent was partly evaporated and the residue poured onto crushed ice. An oil formed which soon solidified. The solid was washed with water, dried and crystallized. Yields were 50–75%.

The biological tests were carried out[11] by methods described previously[12,7]. In in vitro tests the compounds showed very low activity against the test organisms, *Alternaria brassicicola, Botrytis cinerea, Septoria nodorum* and *Uromyces fabae*. The concentration required to inhibit germination completely was above 1000 ppm in all cases. As protectant sprays for the control of wheat powdery mildew, *Erysiphe graminis*, only compound (III) showed much activity at an application rate of 100 ppm. It gave 47% disease control. The results of tests for systemic activity by root application against *E. graminis* on wheat growing in sand are given in Table II. It is evident that compound (III) is a very effective systemic fungicide for the control of wheat powdery mildew giving complete disease control at 5 ppm after 7 days and over 98% disease control after 23 days. Compounds (IV) and (VI) also show good systemic activity while compound (V) is slightly active. The compounds were much less effective by soil application presumably due to their absorption on soil constituents (cf.[7]).

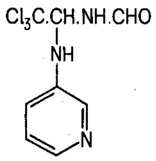


I



$Cl_3C.\underset{R}{CH}.NH.CHO$

II



III

Table II. Systemic activity against *E. graminis* on sand-grown wheat seedlings 7 days after treatment

Compound number	Infection grade (% of control) ppm 100	50	25	10	5
III	0+[a]	0	0	0	0
			0[b]	< 2[b]	< 2[b]
IV	14	43	93	–	–
V	75	96	100	–	–
VI	0+	7+	43	–	–
I	0	0	0	0	0
			0+[b]	8[b]	40[b]

[a] + Indicates phytotoxicity. [b] After 23 days.

The discovery of systemic activity in compounds (III)-(VI) considerably extends the range of compounds of type (II) now known to possess systemic fungicidal properties. It reinforces the belief[7,8] that the antifungal effects are associated with the chemical grouping CX_3–CH. NH.CHO(X=Br or Cl). Chemical modifications on the –NH.CHO grouping have so far resulted in substantial loss of systemic fungicidal activity[7,8]. It is interesting to note that like several of its systemically active relatives[7–9], compound (III) is a poor fungistat and has mediocre protectant activity. The reasons for this behaviour are not yet clear but it suggests that the systemic activity may be due to conversion in vivo to a more active entity or to some indirect mechanism.

Summary. The systemic fungicidal activity of a new formamide, *N*-[2, 2, 2-trichloro-1-(3-pyridylamino)ethyl]-formamide, is reported.

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[11] Dr. G. A. CARTER of the Agricultural Research Council, Plant Growth Substance and Systemic Fungicide Unit, Wye College, University of London kindly carried out the biological tests. Prof. R. L. WAIN, Director of the Unit, is warmly thanked for his help in arranging the tests and for research discussions.

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The Oxindole Alkaloids of *Vinca elegantissima* Hort.[1]

The isolation of reserpinine and an oxindole alkaloid designated as elegantine was reported earlier[2] from *Vinca elegantissima* Hort. var. *Vinca major* Linn. (Apocynaceae). The compound elegantine has now been found to be identical with isomajdine (**1**)[3]. An authentic sample of the latter not being available, the identity was established by comparison of their physical and spectroscopic data and through isomerisation of elegantine to majdine (**2**)[3], which was identified by direct comparison.

Further investigation of *V. elegantissima* has led to the isolation of majdine itself, vincamajoreine[4] and lochnerine[5]. In addition, two new oxindole bases named elegantissine (**3**), amorphous, $[\alpha]_D + 3.4°$ ($CHCl_3$) and isoelegantissine (**4**), m.p. 190°, $[\alpha]_D + 5.7°$ ($CHCl_3$) could be isolated in very low yields. The mass spectra (M^+ 428) of both of them were almost superimposable to that of **1** and their IR-spectra were very close to those of **1** and **2**; the 4 compounds exhibited almost identical UV-spectra. The