Brit. J. Pharmacol. (1961), 17, 286–296.

INFLUENCE OF DICHLOROACETYLATION ON THE ANTIMICROBIAL ACTIVITY OF CHLORAMPHENICOL DERIVATIVES AND OF VARIOUS AMINES

BY

W. LOGEMANN, L. ALMIRANTE, S. GALIMBERTI AND I. DE CARNERI

From the Departments of Organic Chemistry and Microbiology, Research Institute of Carlo Erba S.p.A., Milan, Italy

(*Received June* 28, 1961)

The dichloroacetyl group, one of the functional groups responsible for the antibacterial activity of chloramphenicol, has been inserted into various amines, and the dichloroacetamido-derivatives obtained have been studied against *E. histolytica* EdM, *Candida albicans* ATCC 2091, *Saccharomyces cerevisiae* ATCC 7921, *Aspergillus niger* NRRL 3, *Trichomonas vaginalis* and *Mycobacterium tuberculosis* $H_{37}Rv$ and ATCC 607. Among the various chlorophenoxamide analogues, only *N*-(benzothiazol-2-ylmethyl)dichloro-*N*-2-hydroxyethylacetamide shows an activity, *in vitro* and *in vivo*, comparable with that of chlorophenoxamide. The dichloroacetyl group is essential for the high amoebicidal activity *in vivo* of chlorophenoxamide. The dichloroacetamidopropiophenones of known antifungal activity also show marked amoebicidal activity *in vitro*, but they appear to have a different mechanism of action from chlorophenoxamide since the dichloroacetyl group is not indispensable. The high antitubercular activity of some benzothiazole derivatives is not increased by dichloroacetylation.

Attempts at changing the chemical structure and configuration of chloramphenicol, leaving the dichloroacetyl group untouched, have not as yet led to the discovery of compounds with an antibacterial activity equal to that of the natural antibiotic.

Some derivatives, among the large number which have been synthesized and studied, do, however, show other biological activities which are slight or absent in chloramphenicol.

For instance, Long & Troutman (1951) have been able to demonstrate that α-dichloroacetamido-β-hydroxy-*p*-nitropropiophenone, whose structure differs from that of chloramphenicol by the transformation of the secondary alcohol group into a keto-group, with consequent disappearance of a centre of asymmetry, has an antifungal activity (particularly on *Candida albicans*) much greater than that of chloramphenicol itself.

Starting from these facts, we have studied the influence exerted by the dichloroacetyl group on the biological activity of various homogeneous classes of compounds. For this purpose we synthesized the substances classified in Tables 1 to 5.

METHODS

We determined the amoebicidal activity *in vitro* and *in vivo*, also the trichomonacidal, antifungal and antitubercular activities *in vitro*.

The *in vitro* amoebicidal activity was determined by performing microscopical examinations and subcultures of test-tubes with 4 ml. of the dilutions in Pavlova medium, 48 hr after seeding with 40,000 *E. histolytica* trophozoites, strain EdM (de Carneri, 1958a).

The *in vivo* antiamoebic activity was determined on young white rats by performing the examination of the caecum 5 days after injection of 250,000 trophozoites of the virulent Meah strain (Jones, 1946).

The trichomonacidal activity was tested on suspensions of 30,000 *Trichomonas vaginalis* in each ml. of CPLM medium (de Carneri, 1956a, b), reading the results after 24 hr at 37° C.

Antimycotic activity was tested on *Candida albicans* ATCC 2091, *Saccharomyces cerevisiae* ATCC 7921 and *Aspergillus niger* NRRL 3 by performing serial dilutions in Sabouraud's broth, seeding 10,000 micro-organisms/ml. and reading the results after 3 days at 27° C.

For determining the antitubercular activity, two strains of *Mycobacterium tuberculosis* were used: $H_{37}Rv$, of human origin, and ATCC 607, non-pathogenic and of rapid growth. Serial dilutions in Youmans medium were performed, each 4 ml. tube was seeded with 0.04 ml. of wet bacteria, and readings were taken after 14 days and 3 days at 37° C, respectively, the growth at the top and bottom of the tubes being observed.

RESULTS

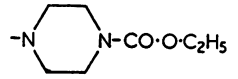
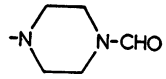
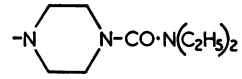
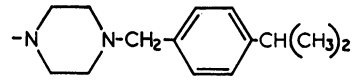
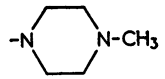
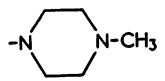
Derivatives of α-dichloroacetamidopropiophenone

Table 1 gives some basic derivatives of α-dichloroacetamidopropiophenone which were new compounds; these were obtained by a procedure already described (Alberti, 1956) consisting of the addition of secondary amines to α-dichloroacetamidoacrylophenones. By this method we prepared a series of substituted piperazines (compounds 1 to 7) and aziridines (compounds 8 to 11). Other compounds were obtained by acid hydrolysis of the aziridine derivatives (compounds 12 and 13) and by addition of isoniazid (isonicotinohydrazide) (compound 14). These propiophenone derivatives, and βp-dichloro-α-dichloroacetamidopropiophenone (compound 15), have considerable activity not only against several fungi (especially *Candida albicans*) but also against *Entamoeba histolytica in vitro* (de Carneri, 1958a, b).

It is well known that the antiamoebic activity of a compound can be exerted either directly on the parasite or through destruction of the bacterial flora on which the amoebae themselves live; but these compounds, whose structure is very similar to that of chloramphenicol, act directly on the parasite. This is shown clearly by their rapidity of action, the amoebae being killed within 1 hr after exposure to the lower effective doses. In addition, a direct amoebicidal action of a chloramphenicol analogue is also demonstrated by the fact that the L-*threo* isomer of chloramphenicol, which has no antibacterial activity, has the same amoebicidal activity as the natural antibiotic (de Carneri, 1956a). Except for compounds 10, 12 and 13, which are only slightly active, the new compounds listed in Table 1 show an amoebicidal activity of about 10 μg/ml. *in vitro*, while *in vivo* (in white rats) they do not display any activity. Studies now in progress show that the amoebicidal activity of compound 15 and of the corresponding acetyl derivatives is identical (10 μg/ml.).

TABLE 1

MINIMAL CONCENTRATIONS (μG/ML.) OF DICHLOROACETAMIDOPROPIOPHENONES OF THE GENERAL FORMULA $R.CO.CH(NH.CO.CHCl_2).CH_2.R'$ REQUIRED FOR COMPLETE GROWTH INHIBITION OF A SERIES OF MICRO-ORGANISMS

Com. pound no.	R	R′	*Entamoeba histolytica* EdM	*Candida albicans* ATCC 2091	*Sacchar. cerevisiae* ATCC 7921	*Asperg. niger* NRRL 3	*Trichom. vagin.*	*Myco. tuberculosis* $H_{37}Rv$	*Myco. tuberculosis* ATCC 607
1	$-NO_2.C_6H_4-$		12·3	7·8	15·6	31·2	250	15·6	62·5
2	$p-NO_2.C_6H_4-$		8·2	31·2	31·2	125	500	7·8	125
3	$p-NO_2.C_6H_4-$		8·2	31·2	62·5	250	250	7·8	125
4	$p-NO_2.C_6H_4-$		12·3	31·2	31·2	125	31·2	1·9	15·6
5	$p-Cl.C_6H_4-$		—	31·2	125	>125	—	—	—
6	C_6H_5-		12·3	11·8	31·2	125	500	1·9	15·6

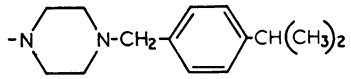
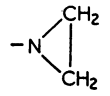
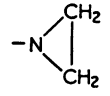
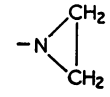
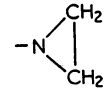
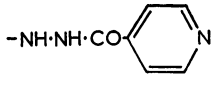
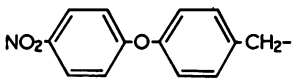
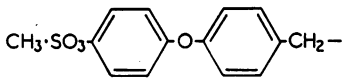
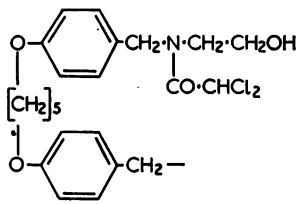
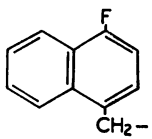
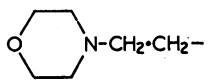
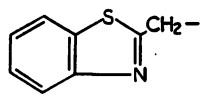
7	*p*-Cl.C$_6$H$_4$–		—	15·6	15·6	31·2	—	1·9	15·6
8	C$_6$H$_5$–		12·3	23·5	46·8	>125	500	0·9	15·6
9	*p*-CH$_3$.SO$_2$.C$_6$H$_4$–		12·3	125	>500	~500	250	—	—
10	*p*-NO$_2$.C$_6$H$_4$–		24·6	15·6	15·6	62·5	125	7·8	31·2
11	*p*-Cl.C$_6$H$_4$–		12	15·6	31·2	>500	125	3·9	15·6
12	*p*-Cl.C$_6$H$_4$–	–NH.CH$_2$.CH$_2$Cl	111	250	>500	>500	>500	31·2	125
13	*p*-Cl.C$_6$H$_4$–	–NH.CH$_2$.CH$_2$.O.SO$_3$H	12·3	15·6	15·6	62·5	125	15·6	62·5
14	*p*-NO$_2$.C$_6$H$_4$–		37	7·8	62·5	125	500	0·9	31·2
15	*p*-Cl.C$_6$H$_4$–	Cl	10	8	16	16	125	8	8

TABLE 2

MINIMAL CONCENTRATIONS (μG/ML.) OF ACETAMIDES WITH THE GENERAL FORMULA $R.N(CO.R'').CH_2.R'$ REQUIRED FOR COMPLETED GROWTH INHIBITION OF A SERIES OF MICRO-ORGANISMS

Compound no.	R	R′	R″	*Entamoeba histolytica* EdM	*Candida albicans* ATCC 2091	*Sacchar. cerevisiae* ATCC 7921	*Asperg. niger* NRRL 3	*Trichom. vagin.*	*Myco. tuberc.* $H_{37}Rv$	*Myco. tuberc.* ATCC 607
16		$-CH_2.O.CO.NH.CONH_2$	$-CHCl_2$	0·6	>500	>500	>500	>500	125	>500
17		$-CH_2OH$	$-CHCl_2$	37	>500	>500	>500	>500	250	500
18		$-CH_2OH$	$-CHCl_2$	>75	>500	>500	>500	>500	125	250
19	$CH_3-[CH_2]_{12}.CH_2-$	$-CH_2OH$	$-CHCl_2$	74	>500	250	>500	125	2·9	15·6
20		$-CH_2OH$	$-CHCl_2$	666	>500	>500	>500	>500	>500	125
21		$-CH_2OH$	$-CHCl_2$	>666	>500	>500	>500	>500	>500	>500
22		$-CH_2OH$	$-CHCl_2$	2·7	—	—	—	—	—	—

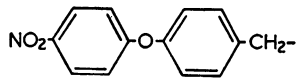
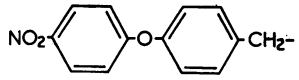
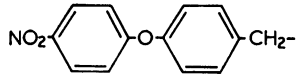
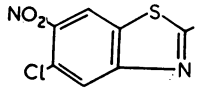
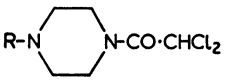
23		$-CH_2OH$	$-CHBr_2$	0·8	—	—	—	—	—	—
24		$-CH_2OH$	$-CH_3$	333	>500	>500	>500	>500	125	500
25		$-CH_2OH$	$-CHCl_2$	0·6	>500	>500	>500	>1,000	>500	>500
26	*p*-$HO.C_6H_4$–	$-CH_2OH$	$-CHCl_2$	666	—	—	—	>500	—	—
27	*p*-$Cl.C_6H_4$–	$-CH_2OH$	$-CHCl_2$	666	>500	>500	>500	>500	250	125
28		–H	$-CHCl_2$	666	—	—	—	>500	—	—
29	*p*-$HO.C_6H_4$–	–H	$-CH_3$	>666	>500	>500	>500	>500	>500	>500

TABLE 3

MINIMAL CONCENTRATIONS (μG/ML.) OF PIPERAZINES WITH THE GENERAL FORMULA  REQUIRED FOR COMPLETE GROWTH INHIBITION OF A SERIES OF MICRO-ORGANISMS

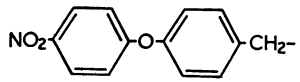
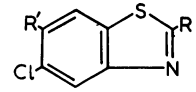
Compound no.	R	*Entamoeba histolytica* EdM	*Candida albicans* ATCC 2091	*Sacchar. cerevisiae* ATCC 7921	*Asperg. niger* NRRL 3	*Trichom. vagin.*	*Myco. tuberculosis* $H_{37}Rv$	*Myco. tuberculosis* ATCC 607
30	$C_6H_5.CH_2.CH_2$–	666	>500	>500	>500	>500	~125	31·2
31	*p*-$(CH_3)_2CH.C_6H_4.CH_2$–	111	>500	>500	>500	>500	62·5	>500
32		222	—	—	—	>500	—	—
33	$CH_3.[CH_2]_{10}.CH_2$–	4·1	>500	>500	>500	>500	0·4	250
34	*p*-$Cl.C_6H_4.CH_2$–	0·3	>500	>500	>500	—	—	—
35	$CH_3.CH_2.CH_2.CH_2$–	222	>500	>500	>500	>500	>500	>500

TABLE 4

MINIMAL CONCENTRATIONS (μG/ML.) OF BENZOTHIAZOLES OF THE GENERAL FORMULA



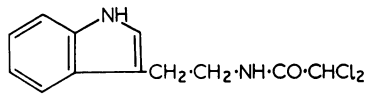
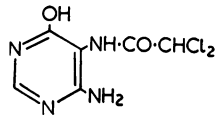
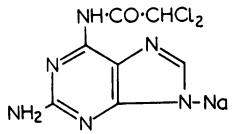
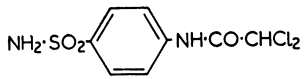
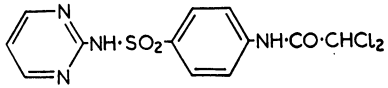
REQUIRED FOR COMPLETE GROWTH INHIBITION *MYCO. TUBERC.*

* Results of a subculture test. In the original test-tubes, a growth on the bottom was repeatedly observed only at 62·5 μg/ml.; the inhibition was complete at higher dilutions of the substance, up to 0·05 μg/ml.; full growth occurs at 0·01 μg/ml.

Compound no.	R	R′	*Myco. tuberc.* $H_{37}Rv$	*Myco. tuberc.* ATCC 607	Compound no.	R	R′	*Myco. tuberc.* $H_{37}Rv$	*Myco. tuberc.* ATCC 607	Compound no.	R	R′	*Myco. tuberc.* $H_{37}Rv$	*Myco. tuberc.* ATCC 607
36	$-SCH_3$	$-NH_2$	0·06	>500	41	$-SCH_3$	$-NH.CO.CH_3$	>500	>500	46	$-SCH_3$	$-NH.CO.CHCl_2$	>500	>500
37	$-SC_2H_5$	$-NH_2$	0·04	3·9	42	$-SC_2H_5$	$-NH.CO.CH_3$	0·1*	93·7	47	$-SC_2H_5$	$-NH.CO.CHCl_2$	0·1*	>125
38	$-NH_2$	$-NH_2$	>500	125	43	$-NH.CO.CH_3$	$-NH.CO.CH_3$	250	>500	48	$-NH.CO.CHCl_2$	$-NH.CO.CHCl_2$	15·6	>500
39	$-NH_2$	$-NO_2$	125	250	44	$-NH.CO.CH_3$	$-NO_2$	~250	>500	49	$-NH.CO.CHCl_2$	$-NO_2$	31·2	31·2
40	$-SH$	$-NH_2$	~500	~500	45	$-SH$	$-NH.CO.CH_3$	~500	>500	50	$-SH$	$-NH.CO.CHCl_2$	62·5	62·5

TABLE 5

MINIMAL CONCENTRATIONS (μG/ML.) OF A SERIES OF DICHLOROACETAMIDES REQUIRED FOR COMPLETE GROWTH INHIBITION OF A SERIES OF MICRO-ORGANISMS

Compound no.	Formula	*Entamoeba histolytica* EdM	*Candida albicans* ATCC 2091	*Sacchar. cerevisiae* ATCC 7921	*Asperg. niger* NRRL 3	*Trichom. vagin.*	*Myco. tuberculosis* $H_{37}Rv$	*Myco. tuberculosis* ATCC 607
51		250	>500	>500	>500	>500	5·8	>500
52		666	>500	>500	>500	>500	>500	>500
53		60	>500	>500	>500	>500	>500	>500
54		>600	>500	>500	>500	>1,000	125	>500
55		>600	>500	>500	>500	>1,000	125	>500

With regard to the antifungal activity, some compounds of Table 1 are completely inactive (9 and 12), while others show marked activity. Among these, compound 10 has been tested as an ointment in clinical practice, and has shown activity against fungal infections, particularly moniliasis. However, it decomposes slowly with time, turning red and losing part of its activity.

Compound 4 shows the most pronounced trichomonacidal activity of this series (31.2 μg/ml.).

Compound 14, an isoniazid derivative, shows, as expected, marked activity against *Myco. tuberculosis.*

Derivatives of chlorophenoxamide and diloxanide

Table 2 contains some new dichloroacetamido-derivatives with structures similar to those of dichloro-*N*-(2-hydroxyethyl)-*N*-[*p*-(*p*-nitrophenoxy)benzyl]acetamide (chlorophenoxamide) (de Carneri, Coppi, Almirante & Logemann, 1960), dichloro-*N*-(2,4-dichlorobenzyl)-*N*-(2-hydroxyethyl)acetamide (chlorbetamide) (Surrey, 1954) and dichloro-*N*-*p*-hydroxyphenyl-*N*-methylacetamide (diloxanide) (Bristow, Oxley, Williams & Woolfe, 1956). These three substances, whose extremely high activity *in vitro* and *in vivo* on *E. histolytica* have been shown by us and by others to exert a highly specific action on *Entamoeba,* are free from any antibacterial activity (de Carneri *et al.,* 1960 ; de Carneri, 1959): unlike the propiophenones previously mentioned, they exert not only an activity *in vitro* but also *in vivo* (de Carneri & Almirante, 1960).

Among the derivatives of Table 2, only *N*-(benzothiazol-2-ylmethyl)dichloro-*N*-2-hydroxyethylacetamide (compound 22) displays an *in vitro* and *in vivo* activity comparable with that of chlorophenoxamide, and this shows the importance of the diphenyl ether linkage to which the dichloroacetamido-group is attached (Logemann, Almirante & de Carneri, 1958 ; and Almirante, de Carneri, Coppi & Logemann, 1960).

The allophanic acid derivative corresponding to chlorophenoxamide itself (compound 16) is active *in vitro,* while it is completely inactive *in vivo.* The replacement of the chlorine atoms by bromine atoms (compound 23) also gives rise to a product with equal amoebicidal activity *in vitro,* but with less activity *in vivo* than chlorophenoxamide.

Compound 24, where the acetyl group has replaced the dichloroacetyl group, is also inactive. The determining influence of the dichloroacetyl group in this type of compound has already been shown in chlorbetamide (Surrey, 1954 ; and Surrey & Rukwid, 1955), where the replacement of this group by the monochloro- and trichloro-acetyl groups led to a reduction in activity.

Of the compounds similar to diloxanide (compounds 26 to 29), the benzothiazolyl derivative (compound 28) is completely inactive. Compounds 26 and 27, having a side-chain in which the methyl group has been replaced by the 2-hydroxyethyl group characteristic of chlorophenoxamide, are also inactive. In these derivatives also the dichloroacetyl group is determinant: replacement with the acetyl group cancels the activity (compound 29).

Compounds shown in Table 2 do not present other particular activities apart from the antitubercular effect of compound 19 which may be due to the presence in this compound of a long aliphatic chain.

N-*Dichloroacetamidopiperazine derivatives*

Table 3 consists of a series of *N*-dichloroacetamidopiperazine derivatives. While compounds 33 and 34 show a high activity *in vitro* on *E. histolytica,* they are inactive in the rats, even at a dosage of 60 mg/kg given *per os* or *per anum* (de Carneri *et al.*, 1960). Compound 33 is slightly active against strain ATCC 607, but shows a strong action against *Myco. tuberculosis* strain $H_{37}Rv$, probably due to the long aliphatic chain of this compound.

Benzothiazole derivatives

Table 4 contains some new benzothiazole derivatives. Our previous studies (Logemann, Galimberti, de Carneri & Coppi, 1960) showed the marked activity of some benzothiazole derivatives against *Myco. tuberculosis.* 6-Amino-5-chloro-2-ethylthiobenzothiazole, for instance, has an antitubercular activity *in vitro* equal to that of isoniazid.

The difference in the antitubercular activity of the amino-derivatives and the corresponding acetyl and dichloroacetyl derivatives of benzothiazole is clearly demonstrated by Table 4. Acetylation of the two most active compounds (36 and 37) leads to distinct reduction of the activity (compounds 41 and 42). Dichloroacetylation of compounds 38 and 39, which are in themselves only slightly active, leads to a marked increase in activity (compounds 48 and 49), but not enough to make these compounds of clinical value.

In contrast, acetylation of compounds 38 and 39 did not lead to any improvement in activity (compounds 43 and 44). None of the compounds included in Table 4 displayed any remarkable activity on *E. histolytica.*

Various compounds

Table 5 shows products obtained by dichloroacetylation of heterocyclic amines of different structures; two dichloroacetylated sulphonamides are also included. These compounds show no special activity.

DISCUSSION

An overall examination of the new compounds in Tables 1 to 5 shows that it is difficult to establish any definite relation between biological activity and presence of the dichloroacetyl group in the compounds as a whole; the presence of the dichloracetyl group determines the activity in only a few cases. It is therefore preferable to compare the activity of various compounds by examining their behaviour, within each single homogeneous class.

The dichloroacetamidopropiophenones, whose antifungal activity is already known, show high amoebicidal activity *in vitro* (Logemann, Lauria, Tosolini & de Carneri, 1958). They have a direct action on amoebae, although they are also antibacterial and can thus act on the parasite by an indirect mechanism through

destruction of the associated flora. Some are also slightly active on *Trichomonas vaginalis*. The dichloroacetyl group is not indispensable for amoebicidal activity, as it can be replaced by other acyl groups without the compounds losing their activity.

In contrast, the dichloroacetamido-group determines the activity of chlorophenoxamide derivtaives. The amoebicidal activity on trophozoites of *Entamoeba* is specific. The group to which the dichloroacetamido-group is linked is also of great importance in these compounds. For instance, in chlorophenoxamide the dichloroacetamido-group is attached to the diphenyl ether group which can be considered most suitable from this point of view, because the chemical stability and low absorption of its derivatives allow it to achieve high concentrations in the intestine for prolonged periods.

We have not been able to confirm the amoebicidal activity observed by Nakamura & Jonsson (1957) in unsubstituted dichloroacetamide; our results showed this compound to be completely inactive both *in vitro* and *in vivo*.

The antitubercular activity of the benzothiazole derivatives is not uniformly increased by dichloroacetylation of the amines.

REFERENCES

ALBERTI, C. G. (1956). Cloramfenicolo-simili: p-nitrofenilendiamino propanoli. *Gazz. Chim. Ital.*, **86**, 240–249.

ALMIRANTE, L., de CARNERI, I., COPPI, G. & LOGEMANN, W. (1960). New amebicides: unsaturated amides related to chlorophenoxamide. *Antibiot. & Chemother.*, **10**, 667–670.

BRISTOW, M. W., OXLEY, P., WILLIAMS, G. A. H. & WOOLFE, G. (1956). Entamide, a new amebicide: preliminary note. *Trans. roy. Soc. trop. Med. Hyg.*, **50**, 182.

de CARNERI, I. (1956a). Azione dei due antipodi ottici del cloramfenicolo e dei treo-1-p-nitrofenil-2-amino-1,3-propandioli enantiomorfi su alcuni protozoi parassiti. *Farmaco ed. sci.*, **11**, 926–933.

de CARNERI, I. (1956b). Conservation of *Trichomonas* in monobacterial cultures. *Amer. J. trop. Med. Hyg.*, **5**, 677–680.

de CARNERI, I. (1958a). Spezifität und Geschwindigkeit der Wirkung zweier verschiedener Reihen von Dichloracetamid-Derivaten auf 3 *Entamoeba*-Arten. *Z. Tropenmed. Parasitol.*, **9**, 32–42.

de CARNERI, I. (1958b). Un gruppo di sostanze a larghissimo spettro di azione: i derivati dell'alfa-dicloracetamido-propiofenone. *Giorn. Mal. Infettive e Parassit.*, **10**, 853–854.

de CARNERI, I. (1959). The use of specific antiamoebic drugs for comparative taxonomic studies. *Trans. roy. Soc. trop. Med. Hyg.*, **53**, 120–121.

de CARNERI, I. (1960). Chemoprophylaxis of amebiasis—with an appendix on the use of Mebinol, a new dichloracetamide derivative. Istituto Carlo Erba per Ricerche Terapeutiche: *Pubblicazioni chimiche, biologiche e mediche*, **3**, 167–201.

de CARNERI, I. & ALMIRANTE, L. (1960). On the reliability of the *in vitro* and *in vivo* determinations for testing anti-amoebic compounds. *Trans. roy. Soc. trop. Med. Hyg.*, **54**, 598–599.

de CARNERI, I., COPPI, G., ALMIRANTE, L. & LOGEMANN, W. (1960). Amebicidal drugs: I, Chlorophenoxamide: an amebicide for chemotherapy and chemoprophylaxis. *Antibiot. & Chemother.*, **10**, 626–636.

JONES, W. R. (1946). The experimental infection of rats with *Entamoeba histolytica*, with a method for evaluating the antiamoebic properties of new compounds. *Ann. trop. Med.*, **40**, 130–140.

LOGEMANN, W., ALMIRANTE, L. & de CARNERI, I. (1958). Antiprotozoari di sintesi derivati del difeniletere. *Farmaco ed. sci.*, **13**, 139–151.

LOGEMANN, W., GALIMBERTI, S., de CARNERI, I. & COPPI, G. (1960). Attività antitubercolare dei benzotiazoli. Istituto Carlo Erba per Ricerche Terapeutiche: *Pubblicazioni chimiche, biologiche e mediche*, **3**, 227–237.

LOGEMANN, W., LAURIA, F., TOSOLINI, G. & de CARNERI, I. (1958). Studio sull'attività microbiologica di alcuni derivati del cloramfenicolo. *Farmaco ed. sci.*, **13**, 129–138.

LONG, L. M. & TROUTMAN, H. D. (1951). Chloromycetin. Synthesis of α-dichloracetamido-β-hydroxy-*p*-nitropropiophenone. *J. Amer. chem. Soc.*, **73**, 481–482.

NAKAMURA, M. & JONSSON, S. (1957). The effect of antimetabolites on the growth of *Entamoeba histolytica*. I, Purine and pyrimidine analogs. *Arch. Biochem. Biophys.*, **66**, 183–189.

SURREY, A. R. (1954). New amebacides. I, The preparation of some N-benzyl-N-hydroxyalkyl-dichloroacetamides. *J. Amer. chem. Soc.*, **76**, 2214–2216.

SURREY, A. R. & RUKWID, M. K. (1955). New amebacides. II, The preparation of some N-alkyl-N-benzylhaloacetamides. *J. Amer. chem. Soc.*, **77**, 3798–3801.