ANTIVIRAL ACTIVITY OF N,N'-DIMETHYL-EPIDITHIAPIPERAZINEDIONE, A SYNTHETIC COMPOUND RELATED TO THE GLIOTOXINS, LL-S88α AND β, CHETOMIN AND THE SPORIDESMINS

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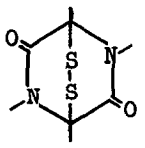
SUMMARY

The gliotoxins and LL-S88α (also named acetylaranotin) have in common the epidithiapiperazinedione moiety (I) and the ability to inhibit the multiplication of RNA viruses. Therefore, a simple compound containing the structural feature (I) was synthesized; it too inhibited the multiplication of RNA viruses.



(I)

INTRODUCTION

The fact that a number of natural products containing the epidithiapiperazinedione moiety (I) have similar biological properties was first noted by Brewer et al., (1966) and the subject was reviewed recently by Taylor (1967). The properties described include inhibition of bacteria, fungi and viruses and also toxicity in animals, plants and tissue cultures derived therefrom.

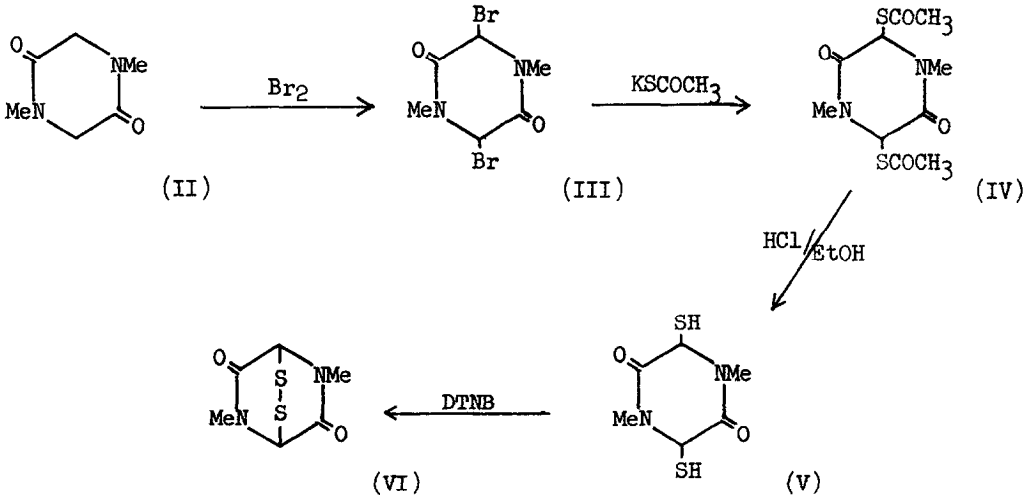
Recently, attention has been focused on the antiviral activities of this group of compounds. Gliotoxin inhibits RNA viruses both in tissue culture and animals; (Rightsel et al., 1964, Larin et al., 1965). It apparently does so by blocking, specifically and irreversibly, the synthesis of viral RNA (Miller et al., 1968a). Another group of compounds, discovered independently at Lilly Research Laboratories (Nagarajan et al., 1968) and these laboratories (Miller

et al., 1968b) and named the aranotins and LL-S88α respectively by the two groups, also possesses antiviral activity. LL-S88α inhibits RNA viruses both in tissue culture and in animals and, like gliotoxin, specifically inhibits viral RNA replication (Miller et al., 1968b). In addition, chetomin, reported to contain the moiety (I) (Brewer et al., 1966), is shown in this paper to inhibit specifically the synthesis of viral RNA (Table I).

Removal of the sulfur atoms from epidithiapiperazinediones results in the loss of biological activity. For example, desulfurization of gliotoxin and sporidesmin abolished their antibacterial activities (Brewer et al., 1966). Also, antiviral activity is lost when the sulfur bridge is removed from gliotoxin or LL-S88α (Table I), or when it is reduced and methylated in the latter compound to give LL-S88β (Table I). Circumstantial evidence therefore points to the epidithiapiperazinedione moiety as the active center of the natural products containing it. The synthesis of a simple compound containing that moiety and its antiviral activity are the subjects of this paper.

METHODS

The scheme of reactions used in the synthesis was as follows:



(II) (III) (IV) (V) (VI)

1,4-Dimethyl-2,5-piperazinedione ("sarcosine anhydride," II) was treated with bromine at 150° in o-dichlorobenzene to give the corresponding

3,6-dibromide III in 70% yield, m.p. 139-143°, ir, lactam carbonyl shifts from 6.02 μ to 5.85 μ on bromination (Analysis: calculated for $C_6H_8N_2O_2Br_2$; C, 24.02; H, 2.69; N, 9.34; Br, 53.27. Found C, 24.16; H, 3.03; N, 8.82; Br, 51.90). nmr ($CDCl_3$), signals at δ = 3.08 (6 H, singlet) and 6.05 (2 H, singlet). Reaction of III with potassium thioacetate at 0° gave the corresponding 3,6-dithioacetate (IV), 95% yield, m.p. 205.5-208°, ir, additional band in carbonyl region at 5.83 μ (thioacetate) (Analysis: calculated for $C_{10}H_{14}N_2O_4S_2$, M.W. 290: C, 41.45; H, 4.83; N, 9.65; O, 22.02; S, 22.05. Found C, 41.55; H, 5.09; N, 10.13; O, 21.54; S, 21.09). ms, molecular ion at m/e 290 and a fragmentation pattern entirely consistent with the structure IV. Hydrolysis of IV in ethanolic HCl gave the corresponding 3,6-dithiol (V) in 64% yield, mp 111-114°, ir, absorption at 5.83 μ absent, band at 3.93 μ (w, SH). (Analysis: calculated for $C_6H_{10}N_2O_2S_2$: C, 34.93; H, 4.89; N, 13.59; O, 15.51; S, 31.08. Found: C, 35.37; H, 4.89; N, 13.58; S, 30.85). nmr ($CDCl_3$), signals at δ = 3.11 (6 H, singlet), 4.96 (2 H, singlet, exchangeable with CD_3OD), 5.08 (2 H, singlet).

Treatment of V with 5,5'-dithiobis-(2-nitrobenzoic acid) [DTNB] at pH 3.5 gave the corresponding 3,6-disulfide (VI) in 72% yield, mp 206-7°, decompn. ir, SH band at 3.93 μ absent, band at 15.2 μ (C-S). (Analysis: calculated for $C_6H_8N_2O_2S_2$, M.W. 204; C, 35.28; H, 3.95; N, 13.72; S, 31.33. Found C, 35.02; H, 3.66; N, 13.55; S, 31.23). ms, molecular ion at m/e 204 and major peak at m/e 140 ($M-S_2$) c.f. ms of gliotoxin (Bose et al., 1968) and LL-S88α (Miller et al., 1968b).

The effects of various compounds on viral and cellular RNA synthesis were measured as follows: Coxsackie A21 virus-infected HeLa S_3 cell suspensions containing ^{3}H-uridine and actinomycin D were prepared by the methods used by Miller et al., 1968a for poliovirus-infected cells. Uninfected HeLa cell suspensions containing ^{3}H-uridine were prepared as described by Miller et al., 1968a. Infected cells were incubated at 37° with graded concentrations of test substances in wells of disposable plastic trays for a five-hour period

beginning 1 hour after infection; uninfected cells were incubated with the test substances at 37° for 4 hours. RNA synthesis was then determined as follows: sodium dodecyl sulfate solution was added to each well (0.2% final concn.) and the contents of the wells mixed for 20 min. at 37°. The well contents were then sampled quantitatively (± 5%) by dipping 1/2" filter paper discs into them for approx. 10 sec. The discs were washed twice for 10 min. in cold 5% TCA, once for 10 min. in ethanol and then air-dried. They were then placed in a vial containing scintillation solution and their radioactivity determined. These methods were described earlier in a preliminary communication (Trown et al., 1967); further details will be reported shortly (manuscript in preparation).

Table I. Inhibition of Viral and Cellular RNA Synthesis by Epidithiapiperazinediones and Related Compounds.

Compound	Concentration (μg/ml) Resulting in a 50% Inhibition(a) of the Synthesis of Coxsackie A21 Virus RNA (A)	HeLa Cell RNA (B)	Inhibition Index B/A
Compound V	0.0003 (± 0.0002)	5 (± 0.4)	15,000
" VI	0.0003 (± 0.0001)	3 (± 0.9)	10,000
Compound IV	0.01	10	1,000
Isodehydrogliotoxin	0.004	2	500
LL-S88α	0.4	100	250
Gliotoxin acetate	0.003	0.8	250
Gliotoxin	0.002 (± 0.0005)	0.5 (± 0.2)	250
Chetomin (30% pure)	0.08	1.3	15
Dethiogliotoxin(b)	60	100	2
Dethio-LL-S88α	40	80	2
LL-S88β	45	100	2
Compound II	> 100	> 100	-

(a) 50% Inhibition concentrations were determined from curves obtained by plotting the results obtained with two-fold dilutions of the test compounds. Each point was the mean of duplicate assays. Results for gliotoxin and compounds V and VI are the averages of 3 separate determinations. All other results are single determinations made with gliotoxin as reference.

(b) Prepared as described by Johnson and Buchanan (1952) and purified by silica gel chromatography.

Compounds IV - VI also inhibit the multiplication of five strains of rhinovirus in conventional tissue culture tests as shown in Table II.

RESULTS AND DISCUSSION

Compounds IV, V, and VI all inhibit viral RNA synthesis at concentrations considerably below those required to inhibit cellular RNA synthesis (Table I).

Compound VI, the 'nucleus' of the gliotoxins, sporidesmins, aranotin and its acetate (LL-S88α) and chetomin, with an inhibition index of 10^4, has one of the highest activities and inhibition indexes of all compounds tested thus far in this antiviral screen (Trown et al., 1967). The activity of IV and V may be explained by intracellular conversion of IV to V followed by oxidation of V to VI. Alternatively V may be the active compound to which VI could be converted by intracellular reduction. Preliminary evidence from derivatives of natural products containing the moiety (I) indicate that the disulfide is the active form.

Table II. Inhibition of the Multiplication of Rhinoviruses by Compounds IV - VI.

Compound	Maximum† Tolerated Concentration (μg/ml)	Minimal Inhibitory Concentration* (μg/ml) against Rhinovirus				
		2060	HGP-5	1200	1059	1734
IV (-3,6-dithioacetate)	4	0.5	0.25	N.T.	N.T.	N.T.
V (-3,6-dithiol)	4	2	N.T.	N.T.	N.T.	N.T.
VI (-3,6-disulfide)	0.25	Inactive	< 0.06	0.06	< 0.06	0.06

† WI-38 cells were used in these tests.

* Denotes concentration at which 50% of the cell sheet remains when the control cells are completely destroyed by the virus.

N.T. denotes not tested.

Compound VI also inhibits the formation of plaques by rhinovirus HGP-5 in HeLa cells; compounds IV and V were not tested. No antiviral activity has been observed in animals thus far.

It may be concluded that the epidithiapiperazinedione moiety is the part of the molecule responsible for the antiviral activities of the gliotoxins, chetomin and LL-S88α (acetylaranotin).

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REFERENCES

Bose, A. K., Das, K. G., Funke, P. T., Kugajevsky, I., Shukla, O. P., Khanchandani, K. S., and Suhadolnik, R. J., J. Amer. Chem. Soc., 90, 1038, (1968).

Brewer, D., Hannah, D. E., Taylor, A., Canad. J. Microbiol., 12, 1187, (1966).

Cosulich, D. B., and van den Hende, J. H., (1968). J. Amer. Chem. Soc. (in press).

Johnson, J. R., and Buchanan, J. B., J. Amer. Chem. Soc., 75, 2103, (1953).

Larin, N. M., Copping, M. P., Herbst-Laier, R. H., Roberts, B., and Wenham, R. B. M., Chemotherapia, 10, 12, (1965).

Miller, P. A., Milstrey, K. P., and Trown, P. W., Science, 159, 431, (1968a).

Miller, P. A., Trown, P. W., Fulmor, W., Morton, G. O., and Karliner, J., (1968b). Biochem. Biophys. Res. Comm. (in press).

Nagarajan, R., Huckstep, L. L., Lively, D. H., DeLong, D. C., Marsh, M. M., and Neuss, N., J. Amer. Chem. Soc., 90, 2980, (1968).

Rightsel, W. A., Schneider, H. G., Sloan, B. J., Graf, P. R., Miller, F. A., Bartz, Q. R., Ehrlich, J., and Dixon, G. J., Nature, 204, 1333, (1964).

Taylor, A. in "Biochemistry of Some Foodborne Microbial Toxins," R. I. Mateles, and G. N. Wogan, Eds., The M. I. T. Press, Cambridge, Mass., 1967.

Trown, P. W., Brindley, K. P., and Miller, P. A., Proc. Vth Int'l. Congress Chemotherapy, Vol. II (1), 7, (1967).