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170. Tyunosin Ukita*¹ and Den'ichi Mizuno*² : *In vitro* Screening of Tricarbonylmethane and Related Compounds for their Anti-tumor Effect by Cylinder Agar Plate (CAP) Method.

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The *in vitro* antibacterial activity of a series of compounds having a tricarbonylmethane group in their structure has been reported by Ukita, *et al.*^{1~11)} From the results of these researches, the tricarbonylmethane group in an alicyclic structure was found to be essential for the antibacterial activity.

The present paper describes the anti-tumor activities of the compounds which have this type of atom grouping and related structures screened by the cylinder agar plate (CAP) method.¹²⁾ The compounds can be classified into four structural types; 3-substituted 4-hydroxycoumarin, 3-substituted 4-hydroxycarbostyryl, 2-substituted 5,5-dimethyl-1,3-cyclohexanedione, and 3-substituted triacetic acid.

Experimental

Antidehydrogenase Test by CAP Method¹²⁾—The CAP method, which is based on the inhibition of the dehydrogenase activity of tumor cells, was applied to test anti-tumor activity of the test compounds against three strains of tumor cells, Ehrlich ascites carcinoma, sarcoma-180, and Yoshida sarcoma. The activities are represented by the diameter of inhibition zone (in mm.) and the test compounds which gave larger diameter than 20 mm. against at least one of the three strains or 15 mm. against at least two of the three strains are taken arbitrarily as active in the following discussion.

Materials used—Seventy synthesized compounds (Nos. 1~70) were tested. The syntheses of these compounds have previously been reported by Ukita, *et al.* and the references are as follows: Compounds Nos. 1~8,³⁾ Nos. 9~11, 13, 18, 19, 47, 48, and 69,^{8,9)} Nos. 12, 14~17,⁷⁾ Nos. 20~25,^{5,6)} Nos. 26~39,⁴⁾ Nos. 40~45, 56~64,¹¹⁾ and Nos. 65 and 66.¹⁰⁾

Results and Discussions

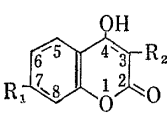

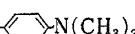


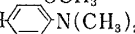





Results are summarized in Tables I, II, III, and IV. Among 3-acyl-4-hydroxycoumarins listed in Table I, four of the aliphatic acyl derivatives from acetyl to valeryl group (Nos. 1~4) showed antidehydrogenase activity somewhat specifically to the two strains of tumor cells. However, no activity was found for the homologs which have longer carbon chain in their acyl group (Nos. 5~8). The activity of this series of compound seemed

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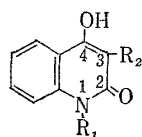
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


TABLE I. Antidehydrogenase Activity of 3-Acyl-4-hydroxycoumarins against Ascites Tumor Cells tested by the Cell Agar Plate (CAP) Method

					
No.	R ₁	R ₂	Ehrlich	Yoshida	S-180
1	H	COCH ₃	15	C	20
2	H	COC ₂ H ₅	11	C	C
3	H	COC ₃ H ₇	25	C	17
4	H	COC ₄ H ₉	20	C	21
5	H	COC ₇ H ₁₅	C	C	C
6	H	COC ₉ H ₁₉	C	C	C
7	H	COC ₁₁ H ₂₃	C	C	C
8	H	COC ₁₅ H ₃₁	C	C	C
9	H	COCH ₂ - 	26	26	25
10	H	COCH ₂ CH ₂ - 	21	20	19
11	H	COCH- 	29	25	26
12	H	COCH=CH- 	O	30	C
13	H	COCH=CH- 	O	O	O
14	H	COCH=CH- 	10	O	C
15	H	COCH=CH- 	C	C	C
16	H	COCH=CH- 	O	O	O
17	H	COCH=CH- 	11	C	10
18	H	CONH ₂	14	C	15
19	H	CONH- 	10	C	13
20	OH	COCH ₃	C	C	C
21	OH	COC ₉ H ₁₉	C	11	C
22	OCOCH ₃	COCH ₃	C	C	C
23	OCOCH ₃	COC ₃ H ₇	16	C	C
24	NO ₂	COCH ₃	30	30	30
25	NO ₂	COC ₃ H ₇	20	C	30

C: Diameter of cup (8 mm.)

TABLE II. Antidehydrogenase Activity of 4-Hydroxycarboystyrils against Ascites Tumor Cells tested by the Cell Agar Plate (CAP) Method



No.	R ₁	R ₂	Ehrlich	Yoshida	S-180
26	H	CHO	25	C	27
27	H	COCH ₃	0	C	C
28	H	COC ₂ H ₅	0	C	C
29	H	COC ₃ H ₇	0	C	15
30	H	COCH ₂ CH(CH ₃) ₂	0	C	C
31	H	COC ₅ H ₁₁	11	C	0
32	H	COC ₉ H ₁₉	0	C	0
33	H	CO- 	C	C	C
34	CH ₃	COCH ₃	11	C	10
35	CH ₃	COC ₃ H ₇	C	C	C
36	CH ₃	COCH ₂ CH(CH ₃) ₂	0	0	C
37	CH ₃	CO- 	C	C	C
38	C ₂ H ₅	COCH ₃	0	C	C
39	C ₂ H ₅	CO- 	11	C	C

C: Diameter of cup (8mm.)

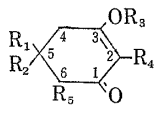



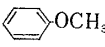
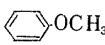







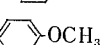




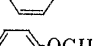




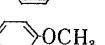
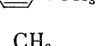





to increase by introduction of nitro group in their 7-position (Nos. 24 and 25).

On the other hand, three aromatic acyl derivatives substituted by phenylacetyl, *p*-dimethylaminophenylpropionyl, and α -dimethylaminophenylacetyl group in the 3-position of 4-hydroxycoumarin (Nos. 9~11) showed remarkable and non-specific activity in this test. Furthermore, it is interesting to note that 3-cinnamoyl-4-hydroxycoumarin derivatives and their analogs (Nos. 12~17), although they can be considered as the dehydrogenated analogs of the above-mentioned compound (No. 10), showed only a slight effect except *p*-hydroxy-*m*-methoxy-cinnamoyl derivative (No. 12) which was active specifically against Yoshida sarcoma cells.

In the case of 4-hydroxycarboystyryl homologs, as shown in Table II, only the 3-formyl derivative (No. 26) showed specific activity to the two of the three strains, whereas the others were not effective despite their structural resemblance to the coumarin derivatives.

The test compounds listed in Table III are the derivatives of 1,3-cyclohexanedione. These compounds are divided into two large groups, i.e. derivatives substituted with 2-phenylcarbamoyl and those with 2-bromo group. Of these two groups, all of the latter showed activity against at least one of the three strains used. On the other hand, the compounds in the former group were active when their 5-position contained no aromatic substituent, thus, the compounds Nos. 46~48 and No. 65 were active. The phenylthiocarbamoyl-substituted derivative (No. 52), which is an analog of the compound No. 47, did not show any large activity. Another type of compounds having bromo substituent

TABLE III. Antidehydrogenase Activity of 1,3-Cyclohexanediones against Ascites Tumor Cells tested by the Cell Agar Plate (CAP) Method

No.						Ehrlich	Yoshida	S-180
	R ₁	R ₂	R ₃	R ₄	R ₅			
40	CH ₃	CH ₃	H	H	H	O	C	15
41			H	H	H	33	C	40
42	H		H	H	H	O	O	C
43	H		COCH ₃	H	H	O	C	O
44	H		H	H	H	C	C	15
45	H		CH ₃	H	H	C	C	17
46	H	H	H	CONH- 	H	20	C	20
47	CH ₃	CH ₃	H	CONH- 	H	17	18	17
48	CH ₃	CH ₃	Na	CONH- 	H	12	26	C
49	H		H	CONH- 	H	O	O	C
50	CH ₃		H	CONH- 	H	O	C	O
51	H		H	CONH- 	H	O	O	C
52	CH ₃	CH ₃	H	CSNH- 	H	13	C	C
53	H		H	CSNH- 	H	O	C	C
54	H		H	CSNH- 	H	O	O	15
55	H		H	CSNH- 	H	O	C	C
56	H	H	H	Br	H	18	C	40
57	H		H	Br	H	13	O	22
58	H		H	Br	H	13	O	32
59	CH ₃	CH ₃	H	Br ₂	H	21	C	40
60	H		CH ₃	Br	H	13	C	15
61	CH ₃	CH ₃	H	H	Br	C	15	13
62			H	H	Br	O	O	12
63	CH ₃	CH ₃	CH ₃	H	Br	33	C	40
64			CH ₃	H	Br	25	C	30
65	CH ₃	CH ₃	H	CONH- 	Br	23	20	15
66	CH ₃	CH ₃	H	CONH- 		15	C	C

C; Diameter of cup (8mm.)

TABLE IV. Antidehydrogenase Activity of Triacetic Acid Derivatives against Ascites Tumor Cells tested by the Cell Agar Plate (CAP) Method

No.	R ₁	R ₂	Ehrlich	Yoshida	S-180
67	H	H	13	17	17
68	H	<div style="text-align: center;"> </div>	0	35	14
69	H	COCH=CH-	24	20	14
70	CO-	CO-	C	O	C

C: Diameter of cup (8mm.)

in their 6-position instead of 2-position were active when its 3-carbonyl group was converted to enol-methylate (Nos. 63 and 64). In the case of 2-phenylcarbamoyl-5,5-dimethyl-1,3-cyclohexanedione, bromine in its 6-position seemed to give no large influence on the activity (No. 65) of the parent compound (No. 47), while substitution with piperidyl group in its 6-position, which was formerly found to have remarkable *in vitro* antibacterial activity against *Escherichia coli*, did not show a very strong activity in this test (No. 66).

Of the four derivatives of triacetic acid listed in Table IV, all compounds except the one which has enol ester group in its 4-position were found active.

Summary

Four groups of compounds which contain an alicyclic tricarbonylmethane grouping, 3-acyl-4-hydroxycoumarins, 3-acyl-4-hydroxycarbostryls, 2-substituted 1,3-cyclohexanediones, and 3-substituted triacetic acid derivatives were synthesized and their antidehydrogenase activity was tested against three kinds of ascites tumor cells, Ehrlich, Yoshida, and S-180, by the cylinder agar plate (CAP) method. From the results obtained, the relationship between the activity and structure of the compound was discussed.

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