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Antibacterial Activity of d-Usnic Acid and Related Compounds on *M. tuberculosis.*

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Usnic acid, a lichen acid isolated over a century ago, has been the subject of active study at various schools of chemistry in respect to structure. That it is a promising antibiotic particularly antagonistic to *M. tuberculosis in vitro* and *in vivo* was discovered by Marshak and has since been confirmed *in vitro* by Stoll, Brack and Renz, Barry and more recently by Shibata and Ukita.[1-4]

The excellent antibacterial characteristics of usnic acid are somewhat offset by its poor water solubility which leads to difficulty in administration to animals and a considerable degree of toxicity. It is therefore of interest to ascertain whether or not these drawbacks could be counteracted by suitable operations on the molecular architecture of usnic acid. Accordingly, a series of derivatives and degradation products of d-usnic acid have been prepared in the hope that one or other of these compounds (No. 2-10, No. 15-18 and No. 21) might possess more advantageous pharmacological and antitubercular properties than usnic acid itself; these could conceivably arise in the animal organism as a result of the customary detoxification mechanisms. More specific attempts to prepare derivatives which

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1 Marshak, A., *U. S. Public Health Repts.*, 1947, **62**, 3.

2 Shibata, S., and Ukita, T., *Japanese Med. J.*, 1948, **1**, 152.

3 Stoll, A., Brack, A., and Renz, V., *Experientia*, 1947, **3**, 115.

4 Barry, V. C., *Nature*, 1947, **160**, 800.

TABLE I.

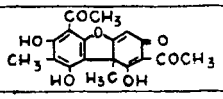
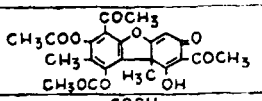
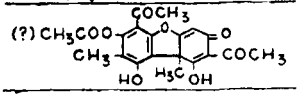
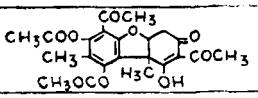
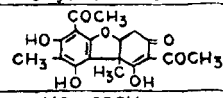
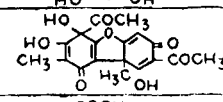
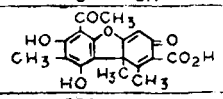
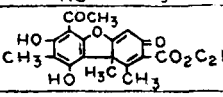
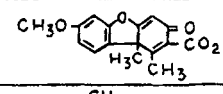
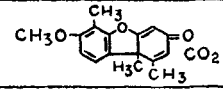
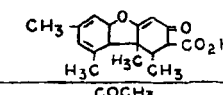
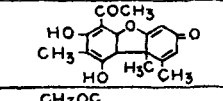
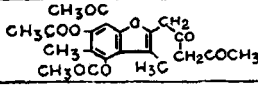
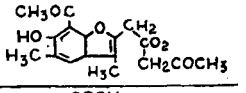
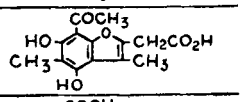
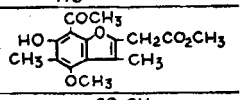
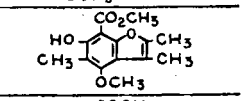
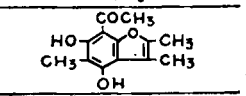
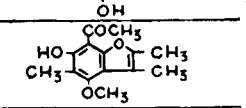
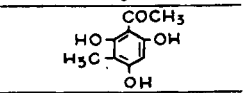
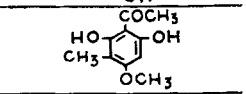
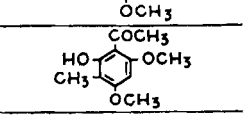
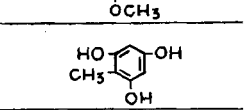
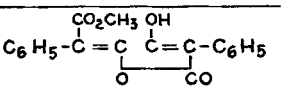
Serial No.	Substance	Structural formula where known in literature	Solubility	Growth of *M. tuberculosis* H 37 Rv — 0 means no growth — Days after inoculation	Drug conc. in μg/cc 20	10	2	0.5
0	(Control)			10	4	4	4	4
				15	4	4	4	4
				20	4	4	4	4
1	*d*-Usnic Acid		c	10	0(0)	0(2)	4(4)	4(4)
				15	0(0)	0(3)	4(4)	4(3)
				20	0(0)	0(3)	4(4)	4(3)
2	rac-Usnic Acid	,,	c	10	0	0	3	4
				15	0	0	4	4
				20	0	0	4	4
3	*d*-Diacetyl Usnic Acid		s	10	0	1	4	4
				15	0	2	4	4
				20	0	2	4	4
4‡	*d*-Monoacetyl Usnic Acid		p	10	0	0	3	4
				15	0	0	3	4
				20	0	0	3	4
5	*d*-Usnamide		p	10	2	3	4	4
				15	2	3	4	4
				20	3	3	4	4
6	*d*-N_5 Methylusnamide		p	10	4	4	4	4
				15	4	4	4	4
				20	4	4	4	4
7	*d*-Diacetyl Dihydro Usnic Acid		s	10	3	4	4	4
				15	3	4	4	4
				20	3	4	4	4
8‡	*l*-Dihydro Usnic Acid		s	10	0	1	4	4
				15	0	2	4	4
				20	0	3	4	4
9	*d*-Usnonic Acid		s	9	2	4	4	4
				12	2	3	4	4
				18	2	3	4	4
10‡	Usnolic Acid		s	10	3	4	4	4
				15	3	4	4	4
				20	4	4	4	4
11	Ethyl Usnolate		s	9	1	3	4	3
				12	2	2	4	4
				18	2	2	4	4
12†	6'-Methoxy-3',3-dimethyl-2',3'-dihydro-benzofurano (2',3',5,4-)-$\Delta^{2:5}$ cyclohexa-dienone-2-carboxylic Acid		s	9	2	3	3	3
				12	2	3	3	4
				18	2	3	3	3
13†	6'Methoxy-3',7',3-trimethyl-2',3'-dihydro-benzofurano (2',3',5,4-)-$\Delta^{2:5}$ cyclohexa-dienone-2-carboxylic Acid		s	9	2	3	3	3
				12	2	3	3	4
				18	2	3	3	3
14†	3',4',6',3-Tetramethyl-2',3'-dihydro-benzofurano (2',3',5,4-)-$\Delta^{2:5}$ cyclohexa-dienone-2-carboxylic Acid		s	9	3	3	3	3
				12	3	3	3	3
				18	3	3	3	3
15	Decarbousnol		s	10	4	4	4	4
				15	4	4	4	4
				20	4	4	4	4
16	Diacetyl decarbousnic Acid		p	10	4	4	4	4
				15	4	4	4	4
				20	4	4	4	4

TABLE I (Continued)

17	Decarbousnic Acid		p	10 15 20	3 3 3	3 4 4	4 4	4 4
18	Usnetic Acid		s	9 12 18	4(3) 4(3) 4(3)	4(4) 4(3) 4(3)	4(4) 4(3) 4(3)	4(4) 4(4) 4(4)
19†	Methylusnetate-4-methyl ether		p	9 12 18	3 2 2	3 4 3	4 4 4	4 4 4
20†	Methyl 2,3,5-trimethyl-4-methoxy-6-hydroxy cumarone-7-carboxylate		p	9 12 18	3 3 3	3 4 4	4 4 4	4 4 4
21	Usnetol		c	9 12 18	4(1) 4(2) 4(4)	4(1) 4(3) 4(4)	4(4) 4(4) 4(4)	4(4) 4(4) 4(4)
22†	Usnetol-4-methyl ether		p	9 12 18	4 4 4	4 4 4	4 4 4	4 4 4
23†	Methyl acetophloroglucinol		s	9 12 18	4 4 4	4 4 4	4 4 4	4 4 4
24†	Methyl acetophloroglucinol-4-methyl ether		s	9 12 18	4 4 4	4 4 4	4 4 4	4 4 4
25†	Methyl acetophloroglucinol-4,6-dimethyl ether		p	9 12 18	4 4 4	4 4 4	4 4 4	4 4 4
26†	Methyl phloroglucinol		s	9 12 18	4 4 4	4 4 4	4 4 4	4 4 4
27	Diacetyl usnic acid ethoxylate		s	9 12 18	3 3 3	3 3 4	4 4 4	4 4 4
28	Sodium *d*-usnate		s	10(9) 15(12) 20(18)	0(0) 0(0) 0(0)	2(0) 3(1) 3(2)	4(3) 4(4) 4(3)	4(4) 4(4) 4(4)
29	3′-Hydroxy usnanilide		p	9 12 18	0 0 0	2 3 3	4 4 4	4 4 4
30	4′-Hydroxy usnanilide		p	9 12 18	4* 3 3	2 2 3	4 4 4	4 4 4
31	4′-Dimethyl amino-usnanilide		p	9 12 18	4 4 4	4 4 4	4 4 4	4 4 4
32	N,β-Carboxyethyl usnamide		s	10 15 20	1 2 1	3 4 4	4 4 4	4 4 4
33	*l*-usnamido napthalene-5-sulfonic acid		s	10 15 20	4 4 4	4 4 4	4 4 4	4 4 4
34	Vulpinic acid		s	10 15 20	1 2 2	3 3 3	4 4 4	4 4 4

* Suspension slightly turbid due to low solubility of drug. This may account for higher density readings.
† Samples of these were furnished by Prof. Alexander Robertson, F.R.S., to whom our grateful thanks are due.
‡ These differed in melting points from those previously recorded in literature, but gave correct analytical figures.
Solubility: s—solution, c—colloid, p—suspension.

might exhibit desirable solubility characteristics consisted in the condensation of usnic acid with substances bearing hydrophylic groupings. The compounds obtained in this connection are No. 29-33. Furthermore, a few substances possessing some of the structural features of usnic acid, No. 11-14, No. 19, No. 20, No. 22-27 have also been included for the examination of their antitubercular potentialities. A total of 33 compounds, related to usnic acid, have therefore been tested against the tubercle bacillus strain H37 Rv. in Dubos medium, using d-usnic acid for comparison. It appears desirable to report the results so far obtained in view of the recent publication of Shibata and Ukita on similar lines. They are given in Table I which also includes vulpinic acid.

We had hoped that this preliminary inquiry would reveal the minimum structural requirements of the usnic acid molecule essential for activity and the relationship, if any, between chemical constitution and antitubercular action in this group so that further planned progress could be made in the chemotherapy of mycobacterial infections. An examination of the present results, which are qualitatively in agreement with those of Stoll and his associates and Shibata and Ukita, could give rise to numerous generalities of a speculative nature. However, the relationship between structure and activity seems to be particularly obscure in this class of compounds. On the one hand, there is the utter lack of specificity of action of the stereoisomeric usnic acids unlike other optically and physiologically active natural products, and on the other, there is the apparently high degree of specificity of the *entire* usnic acid molecule for antitubercular activity. However, it is gratifying to find that some of the simple derivatives of usnic acid, the mono- and diacetyl usnic acids, dihydrousnic acid, m and p-hydroxyusnanilides, have activities equal to or comparable to that of the original substance. These compounds are now being subjected to further investigation.

Experimental. All tests were made by inoculating 0.1 ml of an 8-10-day-old culture diluted 6 x 10^3 into 5.9 ml of culture medium plus drug in 20 x 125 mm screw cap tubes which were then incubated at 37°C. Dubos liquid culture medium was used.[5,6] The culture tubes were previously selected to have the same optical density in the Coleman spectrophotometer to within 0.5%. Readings of the optical density of the culture in these tubes were then taken at 3, 10, 15, and 20 days after inoculation and compared with the growth of the control cultures grown at the same time.

All substances to be tested were dissolved in hot acetone, filtered thru a Corning U.F. filter into a small quantity of culture medium from which the acetone was then evacuated under reduced pressure. The sodium usnate was also tested by dissolving it in a minimal amount of warm (60°C) 2% Na_2CO_3 before addition to the culture medium. Both methods gave the same results. A few compounds gave clear solutions; the remainder were either colloidal or finely divided particulate suspensions as indicated in the table.

The optical density readings at 10, 15, and 20 days after inoculation were expressed as percent of the control readings and then classified into 4 groups, Group 1 being 1 to 25% of the control reading, Group 2, 25 to 50%, Group 3, 50 to 75% and Group 4, 75 to 100%. Zero indicates no growth as measured by optical density. The results are given in Table I. All tests were made in duplicate. In a few cases the tests were repeated at a later date. The values for these are indicated in parentheses.

Summary. The entire usnic acid molecule is involved in its action against the tubercle bacillus since any major alteration in the molecule markedly reduced or destroyed the activity. This is in marked contrast to the equal activity previously demonstrated for stereoisomeric usnic acids.

It is a pleasure to express our indebtedness to Professor Louis F. Fieser, in whose laboratories the chemical work was carried out, for his advice and encouragement, and also to Miss Blanche Burkhart for her assistance with the bacteriological tests.

[5] Dubos, R., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 361.

[6] Dubos, R., and Davis, B., *J. Exp. Med.*, 1946, **83**, 409.