THE CHEMICAL STRUCTURE OF THE ANTIPROTOZOAL PREPARATION METHYLCHLOROPINDOL

V. I. Zaionts, K. I. Shchedrovich,
M. G. Mints, M. V. Krylov,
A. I. Kirillov, and V. I. Loskot

UDC 615.283:547.824

Methylchloropindol (Klopidol) (I), first proposed as a Coyden-25 premix by Dow Chemical Co. in 1963-1965 to reduce the substantial losses from coccidiosis in industrial poultry raising [1], was proved to be a highly effective coccidiostatic agent, inhibiting seven species of coccidi in chickens and guinea fowl, and possessing low toxicity and an absence of cumulations. The preparation is approved for distribution in the USA. It was resynthesized and tested in the USSR [2], the ChSSR, and, under the name of "rigecoccin," in the Hungarian People's Republic. According to the literature [1, 3, 4], without any experimental basis, the assigned structure of I is 3,5-dichloro-2,6-dimethyl-4-hydroxypyridine. Incidentally, such a concept of the chemical structure, which is reflected in its name with an "ol" ending, is illogical. The established regularities concerning the structure of hydroxypryidines, in particular 2,6-dimethyl-4-hydroxypyridine and 3,5-dichloro-4-hydroxypyridine [5, 6], suggested a preferred pyridone structure of I.

An attempt was made to use a biochemical method in order to determine which of the major possible tautomeric forms of I – pyridone or hydroxypyridine – is responsible for its coccidiostatic acitivity. With this object in mind, we synthesized its N-methyl derivative, 3,5-dichloro-1,2,6-trimethyl-4-pyridone (II), and its O-methylderivative, 3,5-dichloro-2,6-dimethyl-4-methoxypyridine (III). Both methylated forms were tested for coccidiostatic activity in parallel with preparation I. The testing was conducted under strictly controlled laboratory conditions, infecting the chicks with a strain of Eimetia tenella in a dose of 50,000 oocysts. Compounds I, II, and III were introduced with the feed in a dose of 0.0125%. A detailed description of the testing method is given in [7].

Anticoccidiosis index I was equal to 174-214; for II, 142-165; and for III, 135-157. The survival percentage for I was 100; for II, 74-80; for III, 80; and for the control group, infected with coccidia and not given the preparation, 0-40. Thus, both methylated forms II and III possess similar coccidiostatic activity; however, it is less than I. This unexpected and interesting result (close biological effect of two isomeric compounds) can be explained by assuming that in vivo N- and O-demethylation of II and III occurs, respectively. The possibility of such enzymatic processes was reported in [8]. It is not precluded that part of II or III, dissimilated by chicks, cannot succeed in being transformed into active I, as a result of demethylation. As a result, II and III exerted a smaller coccidiostatic effect than I.

The resulting data, though interesting, failed to answer the question about the active tautomeric form of I.

Because of this, we made further studies of the structure of I by spectral methods. At this point we assumed that if a strong shift of tautomeric equilibrium toward one of the isomers is discovered, this isomer is the carrier of coccidiostatic activity. We recorded the IR spectra of I in vaseline oil and in fluorinated oil, and spectra of II and III for comparison were also recorded. The resulting data on absorption frequencies of I-III and their interpretation are given in Table 1. Correlation of absorption bands to some groups was based on [9-12].

Since the dissolution of a substance frequently gives rise to additional opportunity for the formation of tautomers, the UV spectra of I, II, and III were recorded in a methanol solution, which causes a maximum

All-Union Scientific-Research Institute of Bird Diseases, Leningrad. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 7, No. 10, pp. 7-10, October, 1973. Original article submitted August 22, 1972.

TABLE 1. IR Absorption Bands of Methylchloropindol (I), Its N- and O-Methyl Derivatives (II and III), and Its Sodium and Silver Salts (V and VI) in Vaseline Oil

Compound	$\nu_{C=O}$	$\nu_{C=C}$		ν_{N-H}	ν_{C-Cl}	$\nu_{=C-O}$
		1	2*			
	cm^{-1}					
I	1560 (s)	1620 (s)	1400 (m)	3200 (m)	760 (s)	—
II	1535 (s)	1610 (s)	1440 (m)	—	760 (s)	—
III	—	1560 (s)	1440 (m)	—	760 (s)	1338 (s)
V	1560 (s)	1640 (m)	1450 (m)	—	760 (s)	—
VI	1570 (s)	1620 (m)	1490 (m)	—	760 (s)	—

* The band was recorded in fluoro-oil.

Designations: s = strong band; m = medium band.

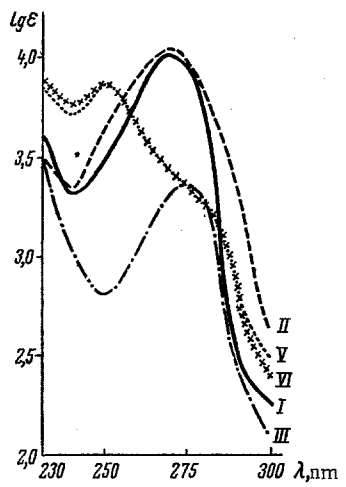


Fig. 1. UV spectra of methylchloropindol (I) and its derivatives: I, II, and III in methanol; V in 0.1 N methanol solution of sodium hydroxide; VI in 8.3 N methanol solution of ammonia.

displacement of tautomeric equilibrium toward the hydroxypyridine form [13-15] (Fig. 1). A comparison of absorption spectra of I, II, and III in the UV region suggests a conclusion concerning the pyridone structure of I not only in a solid state but also in solution (with an accuracy up to 0.1% error as shown by the UV spectrophotometry method) [16].

Having obtained a well-defined proof of the existence of I, exclusively in the form of 3,5-dichloro-2,6-dimethyl-4-pyridone, it can be considered that this structure, being the only one, is coccidiostatically active.

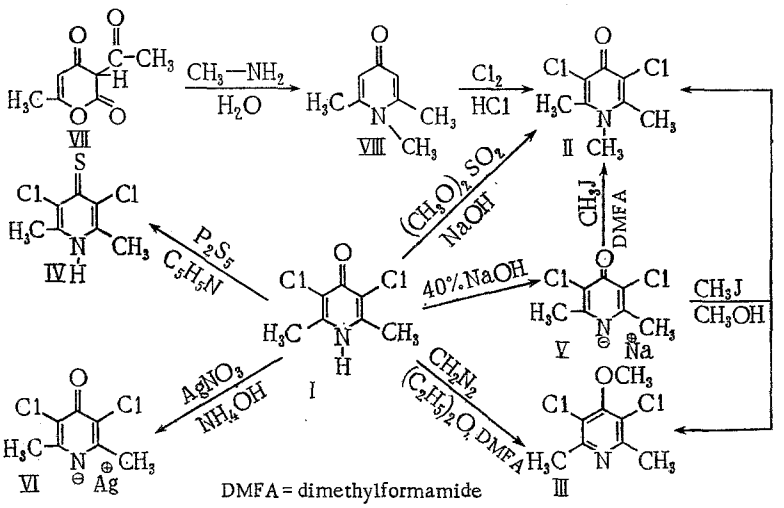
The determination of the true structure of I is not only interesting from a purely chemical standpoint. The pyridone preparation structure resembles the structure of nucleic bases of cytosine, uracil, and thiamine, which enter the composition of DNA and RNA. This analogy may become useful in investigating the mechanism of the activity of the preparation on a molecular level.

The structural closeness of molecule I and nucleic bases suggests that the mechanism of the activity of the preparation consists in the competitive substitution of these bases. Such an assumption is justified by the disappearance of coccidiostatic activity in the synthesized thioanalog of methylchloropindol (V) (anticoccidiosis index, 63-13; survival 13%). The substitution of a sulfur atom for an oxygen atom disrupts the structural resemblance between the preparation molecule and the nucleic base molecule. As a result, the mechanism of competitive displacement is not realized.

Apart from the determination of the structure of I by means of IR and UV spectra, a detailed study was made of the electronic structure of its anion. With this object in mind, a record was made of the UV spectra of I in a methanol solution containing 0.1 g-eq/liter of sodium hydroxide and a silver salt solution of methylchloropindol (VI) in 8.3 N solution of ammonia in methanol (see Fig. 1). First, it was necessary to ascertain the absence of hydrolysis of the sodium salt of methylchloropindol (V) in a medium of 0.05 N sodium hydroxide solution. The spectrum of the anion of I is fairly close to the spectra of I and II, and at the same time it substantially differs from the spectrum of III. This indicates preferentially the pyridone anion structure.. Such a conclusion was verified also by the IR spectra of V and VI. The frequencies of stretching vibrations in V and VI (see Table 1) indicate that, with respect to the double bonds of the C=O group in the methylchloropindol (V, VI) anion, preparations I and II differ little from one another.

The establishment of anion pyridine structure of I is of definite interest owing to the fact that for the unsubstituted 4-pyridone anion, similar to its 2-pyridone isomer, the pyridinolate structure was verified [17, 18].

New syntheses yielding substances for coccidiostatic evaluation and spectral measurements were carried out according to the scheme



EXPERIMENTAL

3,5-Dichloro-2,6-dimethyl-4-pyridone (Methylchloropindol) (I). The synthesis was carried out according to [19-21].

3,5-Dichloro-1,2,6-trimethyl-4-pyridone (II). a) Five g of VIII was dissolved in 150 ml of 3.5% hydrochloric acid solution, then diluted with 700 ml of water, and chlorinated for 3 h. The precipitate was filtered off. Yield, 3.2 g of II (42.8%); 320-322° (from ethanol). Found, %: C 46.76; H 4.33; Cl 34.23; N 7.06. $C_8H_9Cl_2NO$. Calculated, %: C 46.6; H 4.36; Cl 34.5; N 6.80. λ_{max} 270 nm (log ε 4.006). b) Preparation I (1.92 g) was dissolved in 25 ml of 2% sodium hydroxide solution, 0.75 ml of dimethylsulfate added dropwise, and the mixture was heated for 1 h on a boiling water bath; after cooling, the product was filtered off to yield 0.8 g of II (38.6%), mp and data on elementary analysis are similar to those given for method a. A mixture of samples obtained by methods a and b gave no depression in mp. c) A mixture of 2 g of V and 4 ml of methyl iodide in 25 ml of dimethylformamide was boiled for 2 h and then cooled. The product was filtered off to yield 0.8 g of II (57%); mp and data on elementary analysis are similar to those given for method a. A mixture of samples obtained by methods a, b, and c gave no depression in mp. d) A mixture of 2 g of V and 4 ml of methyl iodide in 50 ml of absolute methanol was boiled for 4 h and then cooled. Filtering off yielded 0.4 g of II (21%); mp and data on elementary analysis were similar to those given for method a. A mixture of samples obtained according to methods a, b, c, and d gave no depression in mp. The mother liquor obtained after separating II was treated (see below) to get III.

3,5-Dichloro-2,6-dimethyl-4-methoxypyridine (III). Five g of I in 250 ml of dimethylformamide was treated for 5 h with 5 g of diazomethane dissolved in 100 ml of ether at 20°. The solvent was removed in vacuo by using a water aspirator. Yield, 3 g of III (95%), mp 70° (from methanol). Found, %: C 47.30; H 4.47; Cl 34.02; N 6.94. $C_8H_9Cl_2NO$. Calculated, %: C 46.60; H 4.36; Cl 34.5; N 6.80 λ_{max} 275 nm (log ε 3.367). Mother liquor after separating II in the synthesis according to method d (see above) was evaporated, the dry residue treated with ether, and the extract evaporated. Yield, 0.5 g of III (26%); mp and data on elementary analysis are similar to those given for method a. A mixture of samples obtained according to methods a and b gave no depression in mp.

3,5-Dichloro-2,6-dimethyl-4-thiopyridone (IV). Preparation I (7.7 g) was boiled for 3 h in 75 ml of absolute pyridine with 16 g of phosphorus pentasulfide, and activated carbon was added to the hot solution and filtered. Yield, 5 g of IV (60%); mp 230-232° (from pyridine). Found, %: C 36.74; H 3.32; Cl 34.51; N 6.67; S 14.42. $C_7H_7Cl_2NS$. Calculated, %: C 40.38; H 3.36; Cl 34.13; N 6.73; S 15.38. λ_{max} 260 nm (log ε 3.944); λ_{max} 340 nm (log ε 4.134).

Sodium Salt of 3,5-Dichloro-2,6-dimethyl-4-pyridone (V). Preparation I (10 g) was treated with 50 ml of 40% sodium hydroxide solution, the precipitate filtered off, washed with 20 ml of methanol, and recrystallized from 50 ml of methanol. Yield, 5.6 g of V (50.9%). The product is soluble in water (upon standing a precipitate of I was formed due to hydrolysis), and the solution gives an alkaline reaction. Molecular weight

200 (potentiometric). $C_7H_6Cl_2NNaO$. Calculated 214. λ_{max} nm (log ε 3.866). Attempts to carry out an elementary analysis failed because of incomplete combustion.

Silver Salt of 3,5-Dichloro-2,6-dimethyl-4-pyridone (VI). Preparation I (4 g) was dissolved with heating in 500 ml of 25% aqueous ammonia, and to the mixture was added 30 g of silver nitrate in 50 ml of 25% aqueous ammonia. The mixture was boiled for 40 min, cooled, the precipitate filtered off, washed with water, methanol, and ether. Yield, 6 g of VI (96%). Found, %: C 25.64; H 2.43; Ag 30.04; Cl 22.40; N 4.88; $C_7H_6AgCl_2NO$. Calculated, %: C 28.10; H 2.05; Ag 35.85; Cl 23.70; N 4.68. λ_{max} 250 nm (log ε 3.866). Upon standing, the product darkens.

Dehydracetic Acid (VII). The synthesis was carried out according to the procedure in [20].

1,2,6-Trimethyl-4-pyridone (VIII). Preparation VII (10 g) was heated for 5 h with 50 ml of 25% aqueous methylamine in an autoclave on a boiling water bath and then cooled. Eight g of VIII (97.5%) was filtered off; mp 240-242° (from ethanol) which corresponds to literature data for this substance obtained from 2,6-dimethyl-4-pyrone [22].

IR spectra of I-III, V, and VI were recorded in vaseline oil and in fluror-oil on an IKS-22 spectrometer. UV spectra of solutions of I-III, V, and VI in methanol were recorded on an SF-4a spectrophotometer by using a layer of 10 mm thickness in solution concentrations of 1-2.3 mole/liter. Molecular weight of V was determined by potentiometric titration on LPM-60 M instrument.

LITERATURE CITED

1. U. S. Patent 3206358; Chem. Abs., 63, 9923 (1965).
2. M. V. Krylov, A. A. Tal'drik, A. I. Kirillov, et al., Veterinariya, No. 2, 77 (1971).
3. I. A. Koblova and V. B. Piskov, Khimiya v Sel'skom Khozyaistve, No. 1, 40 (1972).
4. C. S. Wang, I. P. Eeasterby, and N. E. Skelly, Tetrahedron, 27, 2581 (1971).
5. A. R. Katritzky, Chimia (Zurich), 24, 134 (1970).
6. A. Gordon, A. R. Katritzky, and S. K. Roy, J. Chem. Soc. B, 1968, 556.
7. M. V. Krylov, Veterinarya, No. 10, 48 (1969).
8. É. Al'bert, Selective Toxicity [in Russian], Moscow (1971) p. 83.
9. L. J. Bellamy and P. E. Rogasch, Proc. Roy. Soc., London, A, 257, 98 (1960).
10. A. R. Katritzky and R. A. Jones, J. Chem. Soc., 1960, 2947.
11. R. C. Lord, A. C. Marston, and F. A. Miller, Spectrochim. Acta, 9, 113 (1957).
12. A. R. Katritzky and N. A. Goats, J. Chem. Soc., 1959, 2062.
13. A. R. Katritzky, J. D. Rowe, and S. K. Roy, J. Chem. Soc. (B), 1967, 758.
14. E. V. Peresleni, L. N. Yakhontov, D. M. Krasnokutskaya, et al., Dokl. Akad. Nauk, SSSR, Ser. Khim., 177, No. 3, p. 592 (1967).
15. A. Gordon and A. R. Katritzky, Tetrahedron Letters, No. 23, 2767 (1968).
16. A. A. Shishlovskii, in: Applied Physical Optics [in Russian], Moscow (1961) p. 640.
17. H. Speker and H. Gawrosch, Ber. Dtsch. Chem. Ges., 75, 1138 (1942).
18. Yu. N. Sheinker and V. M. Reznikov, Dokl. Akad. Nauk, SSSR, Ser. Khim., 102, No. 1, 109 (1955).
19. British Patent No. 1004941 (1965).
20. A. V. Boese, Ind. Eng. Chem., 32, 16 (1940).
21. V. I. Zaionts, K. I. Shchedrovich, G. S. Volynskaya, et al., Bolezni Ptits, 9 (20), 196 (1973).
22. J. W. Armit and T. J. Nolan, J. Chem. Soc., No. 10, 1931, 3023.