hexane gave pure **1** while the more polar fraction (containing **2**–**2f**) was subjected to preparative gas-liquid chromatography[14] to give pure sample of the trichlorodibromomonoterpene **2**.

Results and discussion. Compound **1**, which accounts for ca. 80% of the total halogenated monoterpenes in A. limacina $[\alpha]_D$ + 4.8 °C (Lit + 5.1 °C)[11], λ_{max} 242 nm (ε, 14, 550 in cyclohexane), (*Lit.* 243 nm)[11], had a MS with M^+ 306, 308, 310, 312 and 314 corresponding to $C_{10}H_{11}Cl_5$ and major peaks at m/e 217, 219, 221, 223 ($C_6H_5Cl_4^+$) and 89.91 (base peak, $C_4H_6Cl^+$). The ^{1}H-NMR of this material also fully conforms with that of 3,4-erythro-7-dichloromethyl-3-methyl-3,4,8-trichloro-1,5(E),7-octatriene (**1**) previously isolated from the pacific red alga Plocamium cartilagineum[11].

Table 1. ^{13}C-NMR chemical shifts for **1** and **2**[a]

	1	**2**
C-1	116.4	116.5
C-2[b]	139.3	139.5
C-3	71.7	71.7
C-4[c]	68.7	69.4
C-5[d]	119.0	123.2
C-6[d]	126.6	124.1
C-7	137.6	137.0
C-8[d]	130.3	132.3
CHX_2[c]	65.5	36.8
CH_3	25.1	25.1

[a] Spectra were determined in [^{2}H] chloroform at 25.20 MHz with a Varian XL-100 Fourier Transform spectrometer operating at both proton-noise decoupling and off-resonance modes; chemical shifts are given in ppm with respect to internal Me_4Si. [b] Assignment based on selective decoupling. [c] The 2 carbons were differentiated by selective decoupling. [d] Assignments may be reversed.

Table 2. 100 MHz ^{1}H-NMR (CCl_4) data for **2**

δ (ppm)/J (Hz)								
CH_3	HA	HB	HC	H-4	H-5	H-6	CHX_2	H-8
1.78 (s)	5.37	5.24	6.02	4.48	6.54	6.58	6.76 (s)	6.30 (s)
	JAC = 16.5			J4,5 ≈ 8[a]				
	JBC = 10.5			J5,6 = 16[a]				

[a] Coupling constants as measured from the spectrum run in C_6D_6, in which the AMX system formed by H-4, H-5 and H-6 was susceptible to first order analysis with signals at 4.14 (H-4), 6.02 (H-6) and 6.16 (H-5) ppm.

The ^{13}C-NMR-data of **1**, collected in table 1, well support this assignment. The second major halogenated monoterpene, **2**, $[\alpha]_D$ −9.7 °C (c, 0.4 in $CHCl_3$), λ_{max} 252 (ε, 0.040 in cyclohexane) did not show a molecular ion, but the presence of fragments at m/e 305, 307, 309 and 311 ($C_6H_5Br_2Cl_2^+$) and m/e 89, 91 (base peak) ($C_4H_6Cl^+$) suggested the molecular formula $C_{10}H_{11}Cl_3Br_2$ from analogy with the fragmentation of the related compound **1**. The ^{1}H-NMR (table 2), also showed a striking resemblance to those of **1** and allowed only 2 gross structures, **2** or the alternative one having a bromochloromethyl group instead of the dibromomethyl group and the bromine instead of the chlorine at C-8, to be assigned to the new monoterpene. Specific location for the halogen atoms followed from ^{13}C-NMR-spectrum (table 1), which was very similar to that of **1** with the only notable exception that the chemical shift of the dihalomethyl carbon is upfield shifted by 28.7 ppm. This is only consistent with a dibromomethyl carbon[15] and in addition replacement of the chlorine by a bromine at C-8 should produce a marked upfield shift (ca. 11–12 ppm) at the α-carbon and a downfield shift (4–5 ppm) at the β-carbon[15]. The stereochemistry of **2** was tentatively assigned by using the ^{1}H-NMR empirical rules developed by Mynderse and Faulkner[11] for the assignments of stereochemistry for Plocamium cartilagineum metabolites. The chemical shift of the methyl signal suggested the threo configuration at carbons 3 and 4 and the chemical shift of proton H-6 (δ 6.58) indicated that the 7,8 double bond has the E geometry.

An examination of the gut contents of A. limacina revealed that it had been eating mainly the red alga Gracilaria verrucosa. Examination of hexane extracts of sun-dried Gracilaria verrucosa and G. compressa did not reveal the presence of halogenated monoterpenes, but **1** accompained by **1a** has been detected by GC-MS in 2 different specimens of Plocamium coccineum collected from different habitats (Naples and Catania). This may indicate that the animals have stored in the digestive gland the metabolites from Plocamium, probably an occasional component of the sea hare's diet, and confirms the endearing ability of the sea hares to concentrate the more interesting compounds from their diet[16]. The algal source of the minor halogenated monoterpenes remain to be discovered, although the failure to locate them in 2 Plocamium specimens might indicate transformations within the digestive gland.

14 2 m × 0.5 cm packed with 10% SE. 30 on silanized chromosorb P 100–200 mesh, operated at 180°; N_2 flowed at 105 ml min^{-1}.

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Anticoccidial riboflavine antagonists

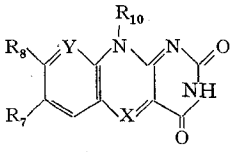
D. W. Graham, J. E. Brown, W. T. Ashton, R. D. Brown and E. F. Rogers

Merck Sharp and Dohme Research Laboratories, P. O. Box 2000, Rahway (New Jersey 07065, USA), 21 March 1977

Summary. 4 types of riboflavine antagonists have broad-spectrum activity in poultry coccidiosis. 5-Deazariboflavine is most effective. 10-Benzyl analogs of riboflavine control intestinal species of coccidia.

Many B-complex vitamin antagonists are effective for the prevention of coccidiosis in poultry and other species. Sulfa-antifol combinations[1], a non-sulfa PABA antagonist[2] and anti-thiamines[3] are used as feed additives for this purpose. Likewise, anticoccidial action has been observed with antagonists of nicotinic acid[4], choline[5] and pyridoxine[6].

Structures and anticoccidial activities



No.	X	Y	R_7	R_8	R_{10}	MED[a] feed level in ppm E. tenella	E. acervulina
1	N	CH	CH_3	CH_3	1-D-ribityl		
2	N	CH	CH_3	CH_3	C_2H_5	---------- [b]	----------
3	N	CH	CH_3	CH_3	n-C_5H_{11}	---------- [b]	----------
4	N	CH	Cl	Cl	$CH_2CH_2CH_2N(C_2H_5)_2$	---------- [c]	----------
5	N	CH	Cl	H	1-D-ribityl	250	250
6	N	CH	CH_3	CH_3NH	1-D-ribityl	60	125
7	N	CH	CH_3	NH_2	1-D-ribityl	60	125
8	N	CH	CH_3	$(CH_3)_2N$	1-D-ribityl	>125[d]	> 250[d]
9	N	N	CH_3	CH_3	1-D-ribityl	125	125
10	CH	CH	CH_3	CH_3	1-D-ribityl	5	50
11	CH	CH	CH_3	CH_3	1-D-ribityl-5-phosphate	10	50
12	N	CH	CH_3	CH_3	3-$ClC_6H_4CH_2$	> 500	125
13	Pargyline					1000	> 1000

[a] The test method employed is described by E. C. McManus, W. C. Campbell and A. C. Cuckler, J. Parasit. *54*, 1190 (1968). [b] See Ball et al.[8]. [c] See Ryley et al.[9]. [d] Compound toxic at this level.

10 years ago E. W. Warren demonstrated that dietary riboflavine (**1**) is required for the normal development of the parasitic coccidia Eimeria acervulina and E. tenella in the chicken[7]. This conclusion was supported by the observation that partial control of an E. acervulina challenge is achieved on administration of the riboflavine analogs 10-ethyl- and 10-n-pentyl-7, 8-dimethylisoalloxazines (**2**, **3**). The activity and accompanying host toxicity of these compounds are reversed with riboflavine[8]. Later 7, 8-dichloro-10-(3-diethylaminopropyl)isoalloxazine (**4**) was found effective against E. brunetti both in tissue culture and bird experiments[9], but since reversal with riboflavine was unsuccessful, the status of **4** as a vitamin antagonist is uncertain. While there has been continued general interest in riboflavine antagonists[10], no further reports have appeared concerning their use in coccidiosis or other protozoan diseases.

During an extended study of this area, we have discovered 4 distinct types of riboflavine antagonists which have substantial broad-spectrum anticoccidial activity, notably 7-chloro-10-D-ribitylisoallaxozine (**5**)[11], 8-methylamino-8-norriboflavine (**6**), 9-azariboflavine (**9**) and 5-deazariboflavine (**10**)[12]. The results of coccidiosis assays on chicks are given in the table[13]. Data are presented as minimum effective feed levels required to control E. tenella, the major cecal species, and E. acervulina, a representative intestinal species. Most remarkable is the finding that 5-deazariboflavine protects birds against E. tenella infection when administered at a feed level of only 5 ppm. Since the riboflavine content of the feed used is 4.8 ppm, the inhibition index of 5-deazariboflavine in this assay is near unity! In a reversal experiment, it was found that, while 10 ppm of the coccidiostat affords protection against an E. tenella challenge when the riboflavine feed level is 9.6 ppm, the effect of 10 ppm of drug is abolished when the vitamin intake is increased to 16.8 ppm. The singular properties of 5-deazariboflavine in flavine enzyme systems have attracted much attention already[14], but these are the first interesting in vivo results on the compound. Its 5'-phosphate derivative (5-deazaFMN, **11**)[14a,1] is a potent coccidiostat also.

Effective control of intestinal coccidia is obtained with vitamin analogs in which the 10-ribityl group is replaced by benzyl or substituted benzyl. The table gives data on 10-(3-chlorobenzyl)-7, 8-dimethylisoalloxazine (**12**), an ef-

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fective agent of this type. While 5'-deoxyriboflavine[15] and riboflavine-5'-sulfate[16] have marginal anticoccidial activity, in general, with the exception of the benzyl compounds, variations from the ribityl group appear unprofitable. Modest activity against E. tenella is observed in tests of N-methyl-N-2-propynylbenzylamine, or pargyline (**13**), a compound which functions by k_{cat} inhibition of flavine-mediated monoamine oxidase[17].

While 8-amino- and 8-dimethylamino-8-norriboflavines (**7**, **8**) were known previously[18,19], the latter as the antibiotic roseoflavine, the 8-methylamino analog (**6**) had not been described when we completed its synthesis, but the compound was reported subsequently as a photolysis product of roseoflavine[20]. Of the 3 amines, **6** is the best coccidiostat, since it is better tolerated than the equipotent 8-amino compound (**7**). Compound **6** [m.p. 307–310 °C; UV_{max} (pH 7) 487 nm (ε 40,500), 306 (8900), 254 (49,900)] and **8** [m.p. 273–277 °C; UV_{max} (pH 7) 506 nm (ε 31,800), 314 (7600), 258 (39,000)][21] were obtained by amination (150fold excess of amine, DMF, 100 °C, 1 h) of 8-chloro-8-norriboflavine[22].

9-Azariboflavine (**9**) [m.p. 255–258 °C; UV_{max} (pH 7) 436 nm (ε 16,700), 312 (4900), 265 (32,500)] was prepared by reacting 2-chloro-5,6-dimethyl-3-nitropyridine[23] with D-ribitylamine, according to the procedure of Israel[24], to give 5,6-dimethyl-3-nitro-2-D-ribitylaminopyridine, m.p. 140–142.5 °C, followed by hydrogenation (Pt, MeOH) of the nitro group and condensation (C_5H_5N, 55 °C, 2 h) of the diamine with 5,5-dichlorobarbituric acid[25].

The 10-(3-chlorobenzyl) analog (**12**) [m.p. 302–305 °C; UV_{max} (MeOH) 440 nm (ε 9800), 347 (8000), 265 (28,200)] was prepared from 4,5-dinitro-o-xylene, 3-chlorobenzylamine and alloxan by a standard three-step procedure[26,27].

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27 All new compounds gave correct elemental analyes.

Aniline hydroxylation in the human red cells

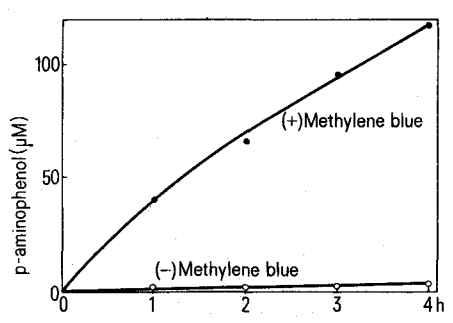
A. Tomoda, T. Yubisui, M. Ida, N. Kawachi and Y. Yoneyama

Department of Biochemistry, Kanazawa University School of Medicine, Kanazawa 920 (Japan) 8 November 1976

Summary. The aniline hydroxylation in human red cells was studied and the hydroxylating activity was much accelerated in the presence of methylene blue.

The hydroxylation of such drugs as aniline has been shown to be catalyzed by microsomal cytochrome P-450 systems in a variety of mammalian tissues since the discovery by Estabrook et al.[1]. However, the contribution of the red cells to this drug hydroxylation has been considered to be negligible because of the deficit of microsome.

Recently Juchau and Symms showed the aniline hydroxylating activity of human hemoglobin[2]. Furthermore Mieyal et al. indicated that hemoglobin can be substituted for P-450 in aniline hydroxylation by microsomal monooxygenase systems[3].



Aniline hydroxylation by red cells in the absence and presence of methylene blue.

On the other hand, human red cells seem to have an intracellular circumstance similar to microsome. The NADH- and NADPH-dependent diaphorases and cytochrome b_5, which are comparable to those of microsome with regard to function and structure, have been recognized in human red cells by many authors[4–6], though it is unclear whether these proteins are derived from the microsome in the stages of the erythroblasts. These results suggest the possibility that red cells are capable of hydroxylating drugs such as aniline.

In spite of these possibilities, the detailed study for aniline hydroxylation by intact red cells has not been reported. This paper deals with the aniline hydroxylation in human red cells and the effect of methylene blue as an activator for the pentose phosphate shunt, which is

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