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CHEMICAL MODIFICATION OF PLANT ALKALOIDS. I. AMINOMETHYLATION OF BARBITURIC ACID DERIVATIVES BY CYTISINE

K. A. Krasnov, V. G. Kartsev, and A. S. Gorovoi

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Reaction of cytisine with 1-mono- and 1,3-disubstituted 5-arylmethylbarbituric acids in the presence of formaldehyde results in aminomethylation of C-5 to form the corresponding 5-cytisylmethylbarbituric acids. The structures of the products are found using PMR spectroscopy and mass spectrometry. 1-Phenyl-5-(2,4-dimethoxybenzyl)-5-cytisylmethylbarbituric acid is obtained as a mixture of two steroisomers in an approximately 2:1 ratio.

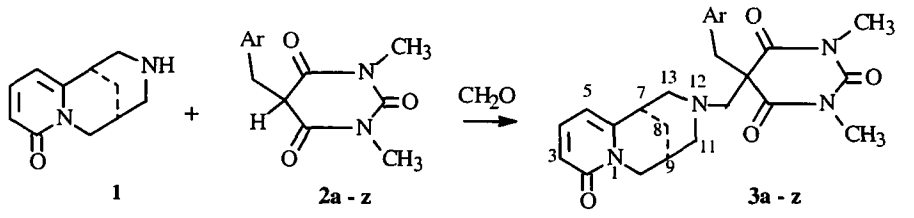
Key words: cytisine, 1-mono- and 1,3-disubstituted 5-arylmethylbarbituric acids, 1,3-dimethyl-5-arylmethyl-5-cytisylmethylbarbituric acids, synthesis.

The alkaloid cytisine (**1**) occurs in seeds of the broom *Cytisus laburnum* L. and *Thermopsis lanceolata* R. Br. It acts on the ganglionic nervous system and is used in medicine as a respiratory analeptic [1]. The distinctive physiological activity is also observed in synthetic N-alkyl- and N-acyl-derivatives of cytisine [2].

One of the most interesting routes for modifying cytisine from the viewpoint of pharmacology is the introduction of barbituric acid, upon which the structures of many medicinal preparations are based, especially those affecting the CNS and the peripheral nervous system [1]. Compounds with distinct anti-inflammatory properties [3, 4] and analgesic and spasmolytic properties [5] are found among 5-aminomethyl derivatives of barbituric acid. N-aminoalkylbarbituric acids [6] also exhibit analogous properties. Some of them are CNS regulators [7].

We developed methods for preparing a new group of potentially biologically active Mannich bases based on cytisine and 5-arylmethylbarbituric acids (**2a-z**). The starting compounds (**2a-z**) were prepared by the new method by reducing the corresponding 5-arylidene derivatives of 1,3-dimethylbarbituric acid with $NaBH_4$ (Table 1). It is known [4-6] that 5-alkyl-; 5,5- and 1,5-dialkyl-; and 1,3,5- and 1,5,5-trialkylbarbituric acids undergo aminomethylation by formaldehyde and secondary amines in alcoholic media (Mannich reaction) to give C- or N-aminomethyl derivatives. The ability of cytisine, a secondary amine, to participate in these reactions has not been investigated.

We found that 1,3-dimethyl-5-arylmethylbarbituric acids (**2a-z**) undergo aminomethylation by cytisine in the presence of formaldehyde to give the corresponding 5-cytisylmethylbarbituric acids (**3a-z**).



1 **2a - z** **3a - z**

Treatment of 1,3-dimethyl-5-benzylbarbituric acid (**2a**) and 1.1 equivalents of cytisine (**1**) (pH 8.0-8.5) in aqueous solution with formaldehyde gives 1,3-dimethyl-5-benzyl-5-cytisylmethylbarbituric acid (**3a**) in 92% yield (Table 2).

1) I. I. Mechnikov St. Petersburg State Medical Academy, 195067, St. Petersburg, Piskarevskii pr., 47; 2) ZAO "Interbioscreen," 142432, Chernogolovka, Moscow District, Institutskii pr. 8. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 152-156, March-April, 2000. Original article submitted February 17, 2000.

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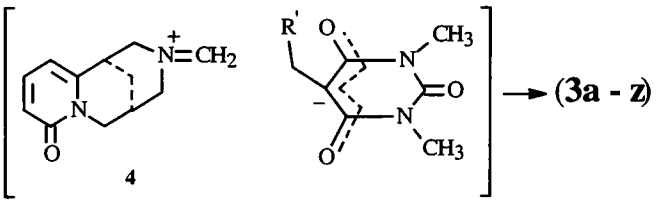
TABLE 1. Yield and Properties of Compounds **2a-z**

Compound No.	Yield, %	mp, °C	R_f($CHCl_3$-ethylacetate, 3:1)
2a	74	116.5-117.5	0.66
2b	71	150-151	0.49
2c	82	99.5-101	0.73
2d	85	86-87	0.60
2e	75	89-91	0.65
2f	88	98-99	0.64
2g	67	95-97	0.66
2h	84	95-96	0.70
2i	71	102-102.5	0.65
2j	69	87-88	0.65
2k	70	60-61.5	0.76
2l	81	101.5-102.5	0.60
2m	90	111-112	0.56
2n	77	120-122	0.59
2o	73	97-98	0.55
2p	70	71-72	0.54
2q	64	164-165	0.47
2r	76	102-103.5	0.52
2s	87	135-136	0.63
2t	69	131-133	0.56
2u	89	179-181	0.65
2v	86	100-101	0.54
2w	75	105-106	0.57
2x	73	110-111	0.55
2y	70	52-53	0.57
2z	85	97-98	0.60

It is interesting that the reaction occurs very fast at room temperature. Most of the product precipitates in the first 10 sec. It was shown that the synthesis of cytisylmethyl derivative **3a** occurs with high yield only in aqueous solutions and requires addition of formaldehyde after mixing the other reagents. This is in contrast with most methods of aminomethylation of barbituric acids in alcoholic media, where the order of mixing of the reagents had no substantial effect [4-6]. A significant quantity of side products forms if this is not done.

The high reactivity of acid **2a** and its analogs under these conditions can be explained by the fact that they undergo the Mannich reaction not in the neutral form but the ionized state. Adding the base cytisine to solutions of these rather strong acids (pKa = 4.7 for 1,3-dimethylbarbituric acid [8] and 3.7-4.0 for 5-benzyl derivatives [9]) completely ionizes them. Treatment of such a mixture with formaldehyde, according to the literature [10], forms from the secondary amine the corresponding imidinium cation (**4**), the reaction of which with the carbanionic acid (**2a-z**) proceeds practically without an activation barrier.

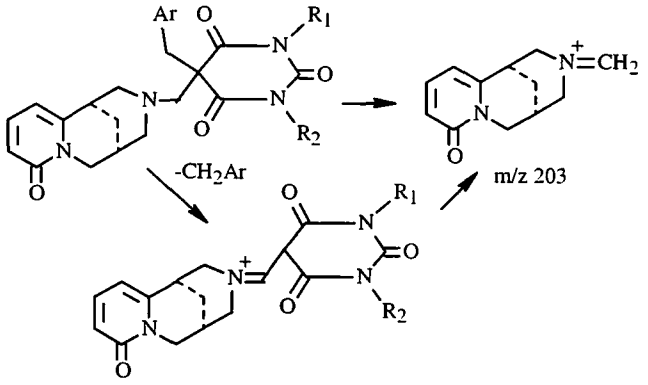


The developed method was used to prepare other 1,3-dimethyl-5-arylmethyl-5-cytisylmethylbarbituric acids (**3a-z**) (Table 3). The structures of these were unambiguously found using PMR spectroscopy (Table 3) and mass spectrometry. Signals in the PMR spectra were assigned to the appropriate groups of the cytisine (Table 3) by taking into account the literature data [11].

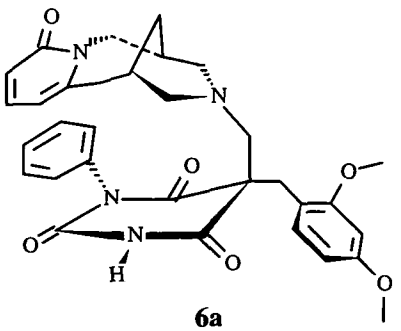
The mass spectra of all compounds (**3a-z**) contain peaks of the corresponding molecular ions with characteristic fragmentation into [M - R] ions with $m/z = 357$ and the methylenecytisinium ion ($m/z = 203$). This enables a general pattern of primary fragmentation of compounds (**3a-z**) by electron impact to be proposed.

TABLE 2. Yield and Properties of Compounds **3a-z**

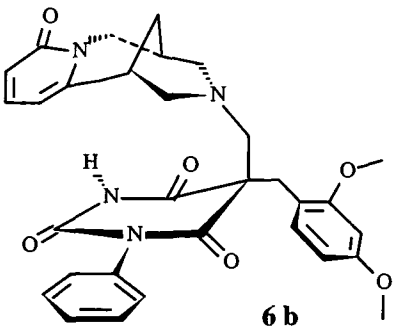
Compound No.	Ar	Yield, %	mp, °C	R_f	M^+	Empirical formula
3a	Phenyl	92	183-184	0.52	448	$C_{25}H_{28}N_4O_4$
3b	*p*-Nitrophenyl	69	214-216	0.45	493	$C_{25}H_{27}N_5O_6$
3c	*p*-Isopropylphenyl	88	174-175	0.63	491	$C_{28}H_{34}N_4O_4$
3d	*p*-Methoxyphenyl	93	126-127	0.45	479	$C_{26}H_{30}N_4O_5$
3e	*o*-Methoxyphenyl	76	219-221	0.47	479	$C_{26}H_{30}N_4O_5$
3f	*p*-Ethoxyphenyl	89	205-206	0.40	493	$C_{27}H_{32}N_4O_5$
3g	*o*-Ethoxyphenyl	70	151-153	0.44	493	$C_{27}H_{32}N_4O_5$
3h	*p*-Isopropoxyphenyl	84	148-150	0.56	507	$C_{28}H_{34}N_4O_5$
3i	*p*-Butoxyphenyl	77	161-163	0.55	521	$C_{29}H_{36}N_4O_5$
3j	*p*-Allyloxyphenyl	91	194-196	0.55	505	$C_{28}H_{32}N_4O_5$
3k	*p*-Octyloxyphenyl	85	120-122	0.45	577	$C_{33}H_{44}N_4O_5$
3l	*p*-Benzyloxyphenyl	72	77-78	0.50	555	$C_{32}H_{34}N_4O_4$
3m	3,4-Dimethoxyphenyl	76	183-184	0.45	509	$C_{27}H_{32}N_4O_6$
3n	2,5-Dimethoxyphenyl	65	184-186	0.51	509	$C_{27}H_{32}N_4O_6$
3o	2,3-Dimethoxyphenyl	72	162-163	0.55	509	$C_{27}H_{32}N_4O_6$
3p	3-MeO-4-benzyloxyphenyl	68	87-88	0.52	585	$C_{33}H_{36}N_4O_6$
3q	3,4,5-Trimethoxyphenyl	61	145-146	0.44	539	$C_{28}H_{34}N_4O_7$
3r	2,3,4-Trimethoxyphenyl	83	160-161	0.49	539	$C_{28}H_{34}N_4O_7$
3s	α-Naphthol	88	233-235	0.57	515	$C_{29}H_{30}N_4O_4$
3t	α-2-Methoxynaphthyl	80	189-191	0.53	529	$C_{30}H_{32}N_4O_5$
3u	9-Anthryl	85	243-245	0.67	549	$C_{33}H_{32}N_4O_4$
3v	2-Thienyl	90	184-185	0.52	455	$C_{23}H_{26}N_4O_4S$
3w	3-Thienyl	84	177-178	0.56	455	$C_{23}H_{26}N_4O_4S$
3x	2-(3-Methylthienyl)	71	194-195	0.51	469	$C_{24}H_{28}N_4O_4S$
3y	2-(5-Methylthienyl)	75	146-147	0.54	469	$C_{24}H_{28}N_4O_4S$
3z	3,4-Methylenedioxyphenyl	81	180-182	0.50	492	$C_{26}H_{28}N_4O_6$



1-Phenyl-5-(2,4-dimethoxybenzyl)-5-cytisylmethylbarbituric acid was prepared from 1-phenyl-5-(2,4-dimethoxybenzyl)barbituric acid (**5**) under analogous conditions. This compound is a mixture of two diastereomers (**6a** and **6b**) owing to the presence of the asymmetric C-5. The explains the presence in the PMR spectrum of a clearly resolved duplicate set of characteristic signals.



6a



6 b

TABLE 3. Chemical Shifts (σ, ppm) and Spin—Spin Coupling Constants (J, Hz) for the Most Characteristic Signals in PMR Spectra of Compounds **3a-z**

Compound No.	2H-8 m, $J_1 = 12.5$ $J_2 = 2.5$	H-9 (br.s)	2H-10 m, $J_1 = 5.0$ $J_2 = 2.0$	H-5 d, J = 6.5	H-3 d, J = 9.0	H-4 dd, $J_1 = 9.0$ $J_2 = 6.5$	2×NCH_3 (3H+3H, s+s)	Other aliphatic protons	Aromatic protons
3a	1.70	2.28	3.70	5.74	6.20	7.15	2.76; 3.02	2.60-3.12(9H)	6.79-7.22m (5H)
3b	1.75	2.30	3.75	5.75	6.30	7.15	2.81; 3.07	2.70-3.17(9H)	7.01d (2H); 7.99d (2H)
3c	1.68	2.32	3.76	5.80	6.30	7.11	2.80; 3.10	1.15 d(6H); 2.60-3.12(9H)	6.70-7.00 d+d (4H)
3d	1.65	2.30	3.72	5.70	6.21	7.10	2.77; 3.04	2.60-3.15(9H); 3.70 s (3H)	6.65m (4H)
3e	1.70	2.30	3.70	5.80	6.30	7.15	2.80; 3.05	2.60-3.10(9H); 3.69 s (3H)	6.70m (3H); 7.16 m (1H)
3f	1.73	2.30	3.82	5.75	6.25	7.15	2.80; 3.05	1.40t (3H); 2.75-3.22(9H); 3.83 q(2H)	6.65m (4H)
3g	1.68	2.25	3.75	5.78	6.22	7.12	2.75; 3.05	1.35t (3H); 2.80-3.23(9H); 3.75 q(2H)	6.60-7.13m (4H)
3h	1.67	2.30	3.75	5.80	6.30	7.10	2.80; 3.10	1.30d (6H); 2.60-3.20(9H); 4.40m (1H)	6.67 d+d (4H)
3i	1.67	2.30	3.75	5.80	6.30	7.15	2.80; 3.10	1.04t (3H); 2.60-3.12(9H); 3.80 q(2H)	6.68 d+d (4H)
3j	1.68	2.25	3.68	5.75	6.23	7.14	2.80; 3.04	2.60-3.12(9H); 4.38d (2H); 5.20 d+d (H_2C =); 5.95m (1H)	6.67m (4H)
3k	1.67	2.28	3.77	5.78	6.25	7.14	2.80; 3.07	0.85t (3H); 2.76-3.20(9H); 3.77m (2H)	6.68m (4H)
3l	1.72	2.30	3.77	5.77	6.30	see ArH	2.80; 3.06	2.58-3.12(9H); 4.98 s(2H)	6.70-7.25m (10H)
3m	1.68	2.30	3.75	5.70	6.20	7.15	2.76; 3.01	2.60-3.10(9H); 3.65 s(3H); 3.75 s(3H)	6.27m (1H); 6.60m (2H)
3n	1.68	2.30	3.75	5.75	6.22	7.15	2.75; 3.05	2.57-3.08(9H); 3.70-3.80 s+ s (6H)	6.35 s (1H); 6.57m (2H)
3o	1.66	2.31	3.77	5.75	6.25	7.16	2.77; 3.04	2.59-3.10(9H); 3.65 s(3H); 3.83 s(3H)	6.30-6.65 (3H)
3p	1.68	2.30	3.78	5.80	6.30	7.10	2.80; 3.06	2.60-3.11(9H); 3.77 s(3H); 5.01 s(2H)	6.30m (3H); 7.35m (5H)
3q	1.70	2.30	3.83	5.75	6.25	7.15	2.78; 3.05	2.57-3.11(9H); 3.80-3.85(9H)	6.00 s (2H)
3r	1.68	2.30	3.75	5.80	6.25	7.15	2.80; 3.10	2.60-3.13(9H); 3.70-3.80(9H)	6.45d (1H); 6.55d (1H)
3s	1.65	2.30	3.75	5.70	6.20	see ArH	2.44; 2.75	2.75-3.44(9H)	7.00-7.70m (8H)
3t	1.67	2.23	3.70	5.73	6.16	see ArH	2.50; 2.90	2.76-3.26(9H); 3.67 s(3H)	6.90-7.40m (7H)
3u	1.68	2.23	3.65	5.67	6.01	6.93	2.00; 2.40	2.85-3.34(9H)	7.41m (4H); 7.93m (4H); 8.28 s (1H)
3v	1.68	2.30	3.81	5.79	6.31	7.20	2.90; 3.10	2.88-3.25(9H)	6.33d (1H); 6.60 d +d (1H); 6.81 (1H)
3w	1.67	2.30	3.73	5.78	6.28	7.15	2.93; 3.09	2.82-3.19(9H)	6.54d (1H); 6.77 s (1H); 7.11d (1H)
3x	1.75	2.30	3.70	5.70	6.21	7.22	2.90; 3.15	2.01 s (3H); 2.90-3.22(9H)	6.62d (1H); 6.90d (1H)
3y	1.70	2.32	3.73	5.80	6.32	7.16	2.91; 3.10	2.30 s (3H); 2.63-3.21(9H)	6.27d (1H); 6.36d (1H)
3z	1.68	2.30	3.75	5.75	see Ar.	7.16	2.83; 3.08	2.60-3.10(9H); 5.86 s (2H)	6.25-6.60m (4H)

The hypothetical structures **6a** and **6b** for these stereoisomers are the most probable taking into account the stereochemical features of related molecules. It is known that C-5 in 5,5-dialkylbarbituric acids deviates from the plane of the pyrimidine ring, imparting to the heterocycle a nonplanar structure. The presence of an aryl substituent on C-5 that is bonded to the pyrimidine ring through a chain of methylene units, for example, in 5-(3-phenylpropyl)barbiturates, leads to the formation of a stable conformation with the aromatic ring oriented parallel to the pyrimidine ring and over it [12].

According to PMR spectra, the content of one of the stereoisomers of **6** is approximately two times greater than the other. This indicates that the aminomethylation of acid **5** by cytisine is stereoselective.

Thus, the developed methods using the Mannich reaction significantly expand the understanding of the chemical reactivity of cytisine and make it possible to prepare new cytisine derivatives that are promising pharmaceuticals.

EXPERIMENTAL

PMR spectra were recorded on an AM-500 Bruker spectrometer at working frequency 500 MHz in $CDCl_3$; mass spectra, on a MX-1303 instrument with direct introduction of samples in the ion chamber at 150°C and ionizing potential 70 eV. The purity of the prepared compounds was monitored using TLC (Silufol UV-254 plates and $CHCl_3$—ethylacetate—acetic acid 3:2:0.1), PMR (Table 3), and elemental analysis.

The purity of the starting compounds was monitored using TLC with $CHCl_3$ and $CHCl_3$—ethylacetate (3:1).

Pharmaceutical grade cytisine isolated from seeds of the broom *Cytisus laburnum* with a purity of >99% was used.

The starting 1,3-dimethyl-5-arylidenebarbituric acids were prepared by the general method [13] from 1,3-dimethylbarbituric acid and the corresponding aromatic or heteroaromatic aldehyde (ArCHO, where Ar is given in Table 2).

1,3-Dimethyl-5-arylmethylbarbituric Acids (2a-z). General Method. 1,3-Dimethyl-5-arylidenebarbituric acid (0.05 mole) in isopropanol (70 ml) and water (10 ml) was heated to boiling. Small portions of $NaBH_4$ (3.7 g, 0.1 mole) were added with stirring. After the exothermal reaction subsided, the mixture was stirred at 70°C for another 20 min.

The mixture was diluted with water (150 ml) and cooled to room temperature. The precipitate was filtered off. The filtrate was acidified with HCl until the pH was 1. The product was separated and dissolved in water (100 ml) with added NH_4OH (25%, 10 ml). The insoluble part was discarded. The solution was treated with HCl. The precipitate was filtered off, washed with water, and recrystallized from aqueous alcohol. The yields and properties of the compounds (**2a-z**) are listed in Table 2.

1-Phenyl-5-(2,4-dimethoxybenzyl)barbituric Acid (5). 1-Phenyl-5-(2,4-dimethoxybenzylidene)barbituric acid was prepared from 1-phenylbarbituric acid and 2,4-dimethoxybenzaldehyde by the literature method [14]. The benzylidene derivative was reduced by the same method as above. Recrystallization from 40% alcohol gave compound **5**, mp 165-167°C, in 55% yield (of theoretical).

1,3-Dimethyl-5-arylmethyl-5-cytisylmethylbarbituric Acids (3a-z). General Method. A mixture of the appropriate compound (**2a-z**, 0.01 mole) and cytisine (2.1 g, 0.011 mole) was heated with alcohol (2 ml) and water (30 ml) and stirred below 45°C until all solids dissolved. The solution was cooled to room temperature and treated with stirring with aqueous formaldehyde (2.1 ml, 0.014 mole, 20%). The mixture stood for 1 h. The precipitate was filtered off, washed with alcohol (40%), and dried. The corresponding derivatives (**3a-z**) were obtained as colorless crystals. Yields and properties are listed in Table 2. PMR data are given in Table 3. Elemental analyses are reported in Table 2.

1-Phenyl-5-(2,4-dimethoxybenzyl)-5-cytisylmethylbarbituric Acid (6). 1-Phenyl-5-(2,4-dimethoxybenzyl)barbituric acid (**5**, 1.76 g, 5 mmole) and cytisine (1.25 g, 7 mmole) were dissolved in water (25 ml) and alcohol (5 ml). The mixture was treated with acetic acid (0.15 ml) to adjust the pH to 7.0-7.5. Aqueous formaldehdye (1.5 ml, 0.01 mole, 20%) was added with stirring. The mixture was held for 1 d at 10°C. The precipitate was filtered off, washed with water and alcohol (40%), and dried. Compound **6** was obtained as white crystals, mp 148°C, yield 63% of theoretical. PMR spectrum ($CDCl_3$, ppm): 1.70-1.85 (2H, m, C-8), 2.30 + 2.35 (1H, br. s + s, C-9), 2.58-3.30 [9H, m, $(CH_2)_3N$ + CH_2Ar + C-7], 3.60 + 3.63 [2.1H + 0.9H, s + s, CH_3O(o), a + b), 3.76 [3H, s, CH_3O(p)], 3.76-4.10 (2H, m, C-10), 5.96 + 6.05 (0.3H + 0.7H, d + d, C-5, a + b), 6.32-6.50 (4H, m, ArH), 6.78 + 6.89 (0.7H + 0.3H, d + d, C-3, a + b), 7.15-7.39 (5H, m, ArH), 7.81 + 9.47 (0.3H + 0.6H, s + s, NH, a + b).

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