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STRUCTURE AND SYNTHESIS OF LEONURINE[a]

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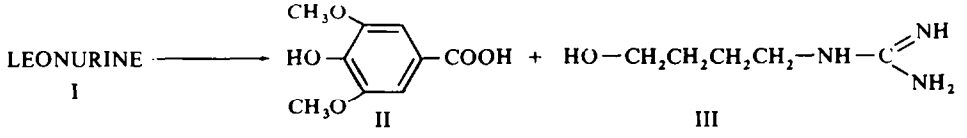
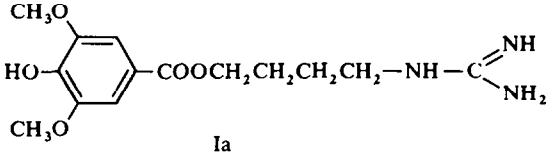
Abstract—The structure of leonurine (I), an alkaloid isolated from *Leonurus sibiricus* L., has been revised as Ia'. Three different methods of synthesis are described.

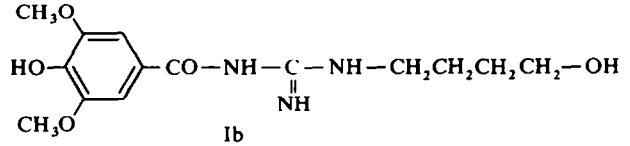
LEONURINE[1], was first isolated from the leaves of *Leonurus sibiricus* L., § in 1930. Later, Hirata *et al.*[2] reinvestigated the alkaloid and concluded that leonurine (I) is composed of syringic acid (II) and 4-guanidino-1-butanol (III) and suggested three possible combinations of these two moieties, namely Ia, Ib, and Ic. They synthesized Ib and it was not identical with the natural alkaloid. Structure Ic was favoured, because the *pKa'* of the alkaloid is 7·9 (basic) in water and probably too low for the *pKa'* of an alkylated guanidino derivative[3] and more likely to correspond to the *pKa'* of an acylguanidino derivative.[2]



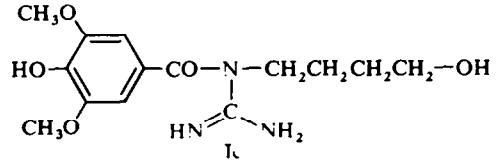
Three possible structures of Leonurine (I)



Ia



Ib



Ic

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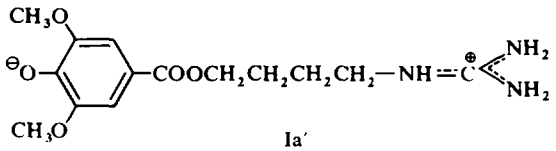
These arguments, however, are not valid since the phenol group in the possible structure Ia may show a lower *pKa'* value than a normal phenol group, if the phenol group in Ia exists in a zwitterionic form as depicted in structure Ia'.



Ia'

In order to clarify these possibilities, *pKa'* measurements were taken in water and in 50% aqueous methanol, respectively. The results are shown in Table 1. This solvent effect[4] clearly indicates that the dissociation group is not basic but acidic in nature. Therefore, the *pKa'* of about 8 for leonurine (I) has originated from the phenolic group and Ia' is the correct structure.

TABLE 1. *pKa'*S OF LEONURINE (I) AND LEONURAMINE (VII)

	in water	in 50% aq. methanol
Leonurine (I)	7·9, above 11	8·7, above 11
Leonuramine (VII)	8·1, 10·4	8·8, 10·2

This structure Ia' was further confirmed by the following syntheses.

The first route, summarized in Fig. 1, established the presence of the ester linkage in the alkaloid.

4-Phthaloyl-1-butanol (V), obtained from 4-chloro-1-butanol (IV), was converted to the corresponding carboethoxysyringate (VI). The compound VI was treated with hydrazine in ethanol, and subsequently with (i) sodium hydroxide and (ii) hydrochloric acid. In this step, the protecting groups of the phenol function, carboethoxyl group, as well as that of the amine function, phthaloyl group, were hydrolyzed. The structure of the product was confirmed as VII by spectroscopic data. It is interesting to note that VII exists as the zwitterionic form (see Table 1) rather than as the phenol amine form. Also this derivative of leonurine has pharmaceutical interests, because a basic hydrolysis of leonurine effects a hydrolysis of the ester part as well as the guanidine part and can not give the compound VII, leonuramine.

Several attempts to transform the amino group in VII into the guanidino group by cyanamide or S-methylisothiourea under various conditions were unsuccessful. The difficulty of the transformation may be attributed to the fact that the amino group in VII exists as the ammonium form and the nucleophilicity of the amino group is decreased. Therefore, the N-nitro-S-methylisothiourea,[5] which may be more easily attacked by a nucleophile, was tried. Indeed, by this reagent the amino group in VII was converted to the guanidino group and the structure of the product was identified by spectroscopic data as VIII, which corresponds to nitroleonurine.

The nitro group in VIII was removed by catalytic reduction, giving a product identical with natural leonurine. The IR spectra of the synthetic compound and the natural leonurine are shown in Fig. 2. As there is no doubt about the presence of

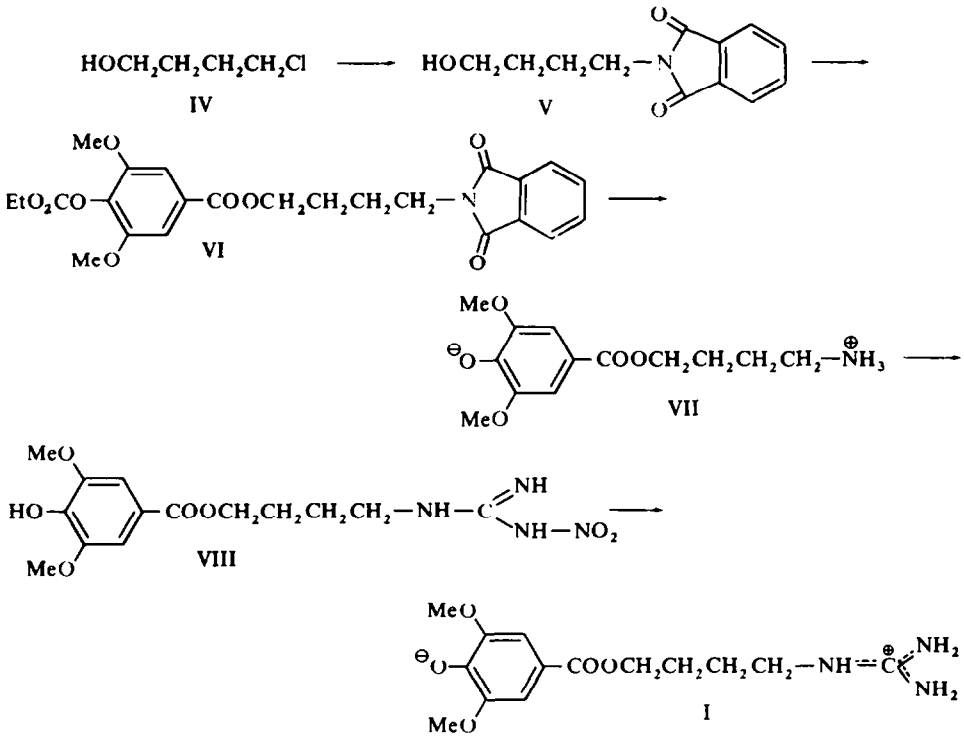


FIG. 1 A route to Leonurine.

the ester linkage in the material synthesized, this also confirms the structure Ia' for leonurine (I).

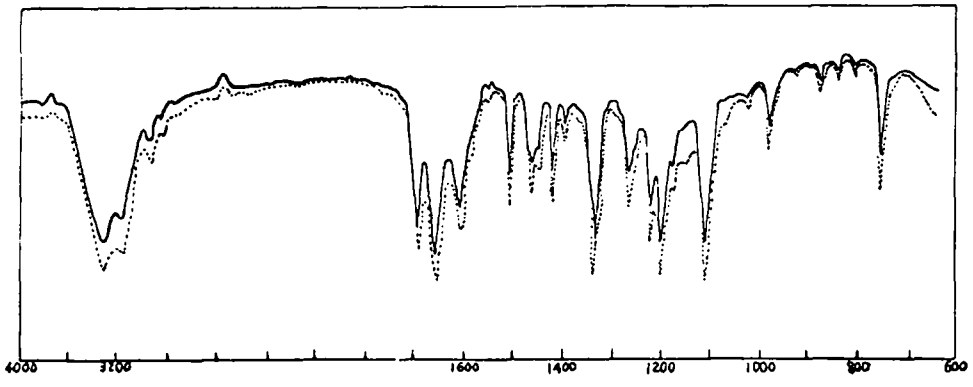


FIG. 2 IR spectra of natural leonurine hydrochloride (- - - -) synthetic leonurine hydrochloride (———) in KBr disc.

The second route to leonurine (I) is shown in Fig. 3.

The carboethoxysyringic acid chloride (IX) was converted to the ester chloride (X) by condensation with tetrahydrofuran in the presence of zinc chloride. The ester chloride (X) was treated with the potassium salt of phthalimide and the resulting

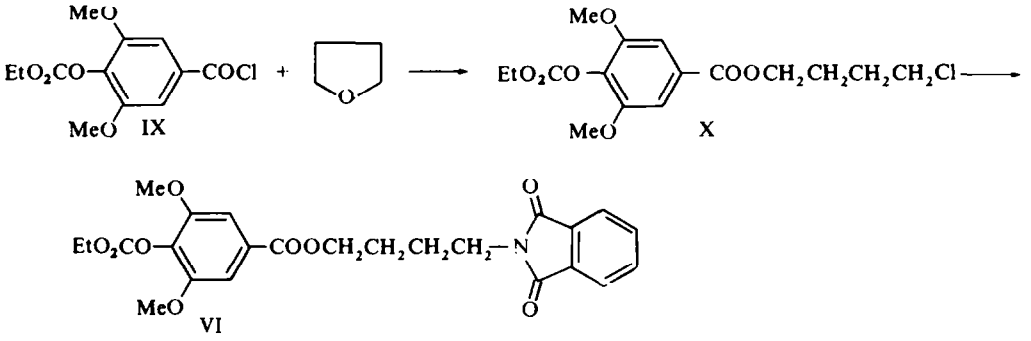


FIG. 3 A route to the Compound (VI)

phthaloyl derivative (VI) was identical with the material described above, making this second route to the natural alkaloid.

The second route to VI gives a better overall yield and is more convenient than the first, but the simplest and best route is shown in Fig. 4.

The known 4-amino-1-butanol (XI)[6] was converted to 4-(N-nitroguanidino)-1-butanol (XII) by the action of N-nitro-S-methylisothiourea at room temperature and without catalyst. The resultant alcohol (XII) was transformed into nitroleonurine (VIII) by simple treatment with carboethoxysyringic acid chloride (IX), followed by sodium hydroxide and successively with hydrochloric acid. The material obtained was identical (IR spectra; m.p.s and mixed m.p.) with the nitroleonurine (VIII) described in the first route.

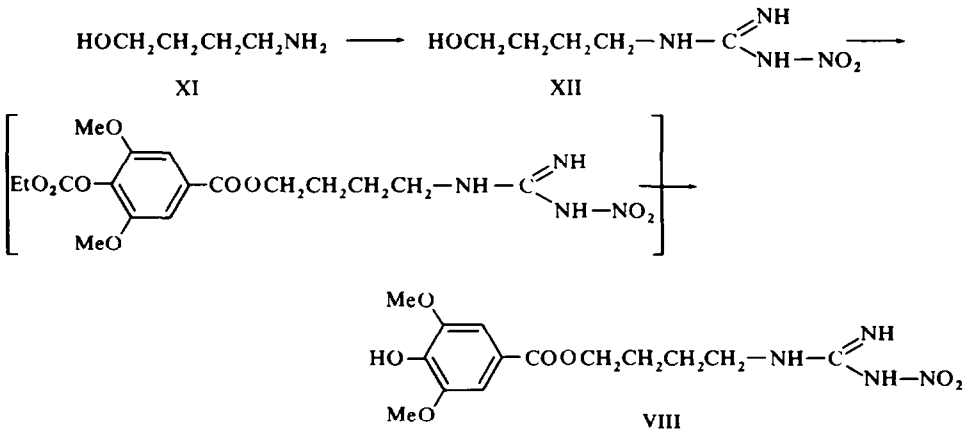


FIG. 4 A Route to Nitroleonurine (VIII)

Usually monoalkylated guanidino derivatives do not give a positive Dragendorff reaction, but as leonurine does give this positive alkaloid test, it was concluded that this is due to the ester linkage of the syringic acid moiety[7] and therefore leonuramine (VII) and leonurine (I) give a false-positive alkaloid test.

As it is possible to get reasonable amounts of leonurine, its derivatives, and its analogs, tests of pharmaceutical activities are in progress.

EXPERIMENTAL

All m.p.s are uncorrected. The UV spectra were recorded on Perkin Elmer 202 Spectrophotometer. The IR spectra were measured with a Nihon-Bunko IR-S Spectrophotometer and with a Nihon-Bunko DS-402G Spectrophotometer. The NMR spectra were taken with a Varian A-60 Spectrophotometer; the chemical shifts are given in ppm relative to an internal TMS standard; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; coupling constants are given in c/s. The mass spectra were determined on Hitachi RMU-6D Mass Spectrometer equipped with a direct inlet system and operating with an ionization energy of 70 eV. The p*Ka*'s were measured by dissolving a few mg of sample in 0·010N HCl (2·0 ml) and titrating it with 0·100N KOH by means of Radiometer TTT-1 pH meter with an automatic recorder. After substracting a blank value, the pH value of the half-neutralized point was taken as the *pKa'*. Unless otherwise noted, the analytical samples were dried over P_2O_5 at 60–80° for 12 hr *in vacuo*.

4-Chloro-1-butanol (IV) was synthesized according to the lit. (*Organic Syntheses* Coll Vol II, page 571).

4-Phthaloyl-1-butanol (V)

The K salt of phthalimide (2·7 g) was added to IV (1·5 g) dissolved in DMF (7·5 ml) and the mixture heated with stirring at 70° for 4 hr. After the reaction mixture had been evaporated to dryness under reduced press, the product was extracted with $CHCl_3$. The extract was washed with 0·1N HCl, and water successively, then dried over Na_2SO_4, filtered, and evaporated to dryness under reduced press. The residual oil was dissolved in benzene, treated with Norit, filtered and evaporated to dryness. The residue was crystallized from benzene-hexane(n), to afford colourless plates (1·4 g; 46%); m.p. 57–59°. (Found: C, 65,89; H, 6·09; N, 6·58. $C_{12}H_{13}O_3N$ requires: C, 65·74; H, 5·98; N, 6·38%); IR: ν^{KBr} 3430, 2960–2850, 1773, 1700 (broad) cm^{-1}: UV; λ_{max}^{MeOH}222 (25,000), 233sh (12,300), 242 (9,000), 297 mμ (ε = 1,600); NMR ($CDCl_3$): 1·68 (5H, one of which disappears on an addition of D_2O; m), 3·76 (4H; m), 7·83 (4H; m).

Carboethoxysyringic acid chloride (IX)

Although IX has been synthesized,[8] a better yield is obtained under the following conditions.

Carboethoxysyringic acid (3 g)[8] was treated with $SOCl_2$ (4 g) at 100° for 40 min. After the excess $SOCl_2$ had been removed under reduced press, the residue was crystallized from ligroin, to afford 2·8 g (88%) of IX, m.p. 71–74°; IR: ν^{KBr} 1766, 1752, 1740, 1606, 1325, 1261, 1216, 1131 cm^{-1}; Mass: M^+.

Carboethoxysyringate (VI) *of 4-phthaloyl-1-butanol*

Method 1. The chloride IX (721 mg) was added below 20° to a soln of V (500 mg) in dry pyridine (10 ml). After the reaction mixture had been stirred at 10–20° for 3 hr, it was poured into 8 ml conc HCl containing ice (25 g). The separated oil solidified slowly and the solid (1·1 g) was crystallized from MeOH, to give 1·0 g (93%) of the ester as colourless prisms, m.p. 124–125°.

Method 2. The K salt of phthalimide (4 g) was suspended in a DMF soln of X (7·9 g in 40 ml) and heated with stirring at 100°. Although the suspension dissolved completely in 70 min, heating was continued for another 20 min. The reaction mixture was partitioned between $CHCl_3$ and water. The $CHCl_3$ layer was washed with Na_2CO_3 aq, dried over Na_2SO_4, filtered and evaporated to dryness under reduced press, to give 10·0 g (97%) of a crude product, m.p. 115–119°. The resultant crystals were recrystallized from MeOH, to afford 7·0 g (68%) of colourless prisms, m.p. 125–126°, identical (IR and mixed m.p.) with VI obtained by the method 1. (Found; C, 61·20; H, 5·39; N, 2·88; $C_{24}H_{25}O_9N$ requires: C, 61·14; H, 5·35; N, 2·97%); IR: ν^{KBr} 1767, 1710 (broad), 1607 cm^{-1}; UV: λ_{max}^{MeOH} 220 (36,200), 233sh (15,200), 242 (13,800), 258 (9,600), 298 mμ (ε = 3,700); NMR ($CDCl_3$): 1·38 (3H; t, J = 7), 1·87 (4H; m), 3·80 (2H; m), 3·92 (6H; s), 4·26 (2H; q, J = 7), 4·43 (2H; m), 7·33 (2H; s), 7·81 (4h; m).

Leonuramine (VII)

Hydrazine monohydrate (0·8 ml) was added to the ethanolic soln of VI and NaOAc (1·60 g and 320 mg in 40 ml) and the reaction mixture was heated under reflux for 3 hr. The resulting crystalline material was washed with water, to give fine crystals, m.p. 202° (dec). These were dissolved in 5% KOH aq and the soln acidified immediately with dil HCl, filtered and neutrallized with $NaHCO_3$aq, to afford a crystalline product (0·75 g; 82%; m.p. 214–215° (dec)). (Found; C, 57·41; H, 7·24; N, 4·96: $C_{13}H_{19}O_5N$ requires: C, 57·98; H, 7·11; N, 5·20%); IR: ν^{KBr} 3600–2200 (broad, strong), 1700sh, 1692, 1630, 1570 cm^{-1}; UV: λ_{max}^{MeOH} 222 (20,900). 279 mμ (ε = 11,000); $\lambda_{max}^{MeOH-NaOH}$ 239 (14,300), 327 mμ (ε = 20,000); NMR(TFA): 2·05 (4H; m), 3·38 (2H; broad), 4·03 (6H; s), 4·56 (2H, broad), 7·50 (2H; s): p*Ka'*: 8·1 and 10·4 in water; 8·8 and 10·2 in 50% aqueous MeOH; Mass: M^+.

Leonuramine (VII) *hydrochloride*

Hydrogen chloride was passed through a suspension of VII in EtOH (0·50 g in 5·0 ml). After the suspension had dissolved, new crystals separated out and the stream of HCl was stopped. The reaction mixture was diluted with ether and the resultant crystals were washed with ether, yielding 0·51 g of the hydrochloride, m.p. 197–198° (recrystallized from MeOH-benzene). The hydrochloride was transformed to the free base on neutralization with $NaHCO_3$ aq.

Nitroleonurine (VIII)

Method 1. Leonuramine (VII) (2·0 g) was suspended in 25 ml EtOH containing 1·0 g NaOH and treated with 1·0 g N-nitro-S-methylisothiourea overnight at room temp. After the reaction mixture had been diluted with water and then slightly acidified with dil HCl, EtOH was removed under reduced press. The resultant viscous soln was scratched and left in a deep freeze. Crystalline nitroleonurine (2·1 g), m.p. 127°, separated out and the starting material (0·4 gr) was recovered on the neutralization of the mother liquor with $NaHCO_3$.

Method 2. The chloride IX (1·3 g) in 17 ml dry pyridine was added dropwise to a pyridine soln of XII (0·8 g in 3 ml) at 5°. After the reaction mixture had been allowed to stand at room temp for 3 hr, it was diluted with ether. The ethereal layer was removed by décantation (this procedure was repeated twice). The remaining oily product was dissolved in dil NaOH aq, treated with Norit and then filtered. The filtrate was acidified with dil HCl, to afford a crystalline product mp 120–127° (1.1 g; 65%). The crude crystals recrystallized from aqueous MeOH as colourless prisms, m.p. 132° (dec), identical with VIII synthesized by the method 1. (Found: C, 44·66; H, 5·96; N, 14·91: $C_{14}H_{20}O_7N_4 \cdot H_2O$ requires: C, 44·92; H, 5·92; N, 14·97%); IR: ν^{KBr} 3640–2800, 1703sh, 1692, 1630, 1605 cm^{-1}; UV: λ_{max}^{MeOH} 222 (24,600), 273 mμ (ε = 24,700); $\lambda_{max}^{MeOH-NaOH}$ 240 (17,900), 271 (17,300), 328 mμ (ε = 19,100); NMR(DMSO-d_6): 1·68 (4H; m), 3·24 (2H; broad t, J = 6), 3·84 (6H; s), 4·26 (2H; broad t, J = 6), 7·25 (2H; s), 7·90 (3H; broad),* 9·28 (1H; broad s).* *: signals disappears on addition of D_2O. *pKa'* (50% aq MeOH): 9·6.

Synthetic leonurine hydrochloride

Nitroleonurine (VIII 1·4 g) was hydrogenated in 60 ml AcOH containing 40 ml 0·2N HCl in the presence of 0·1 g Pd-C (10%). About 4 moles H_2 were absorbed in 40 min. After the catalyst and charcoal had been filtered off, the filtrate was diluted with ether. The resultant crystals were washed with water, to afford 1·1 g (77%) of synthetic I hydrochloride, m.p. 192–193°, identical with the natural leonurine hydrochloride[2] by comparision of IR spectra in KBr disc and by mixed m.p. (Found: C, 45·88; H, 6·87; N, 11·50: $C_{14}H_{21}O_5N_3 \cdot HCl \cdot H_2O$ requires: C, 45·96; H, 6·61; N, 11·49%); IR: ν^{KBr} 3500–2800, 1699, 1664, 1620 cm^{-1}; UV: λ_{max}^{MeOH} 222 (19,800), 278 mμ (ε = 10,800); $\lambda_{max}^{MeOH-NaOH}$ 239 (14,000), 327 mμ (ε = 19,500); *pKa'* (determined on natural leonurine): 7·9 and above 11 (in water); 8·7 and above 11 (in 50% aqueous MeOH).

4-Amino-1-butanol (XI) was synthesized according to Tietze.[6]

Carboethoxysyringate (X) *of* 4-*chloro*-1-*butanol*

Acid chloride IX (5·7 g) was heated at 80° with 1·9 g THF containing 0·4 g $ZnCl_2$. After the chloride had dissolved completely (15 min), the reaction mixture was diluted with benzene, washed with sat NaCl aq and then with $NaHCO_3$ aq. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness under reduced press to give 6·5 g (91%) of an oily product. The product obtained was pure enough for the next step, but for identification purposes, the following purification was carried out. The crude product crystallized slowly on scratching. The resultant crystalline material was recrystallized from n-hexane-benzene; m.p. 38–40° (Found: C, 53·21; H, 5·86: $C_{16}H_{21}O_7Cl$ requires: C, 53·26; H, 5·87%); IR: ν^{KBr} 3000–2800, 1763, 1718, 1608 cm^{-1}; UV: λ_{max}^{MeOH} 213 (29,700), 257 (9,900), 299 mμ (ε = 2,400); NMR($CDCl_3$): 1·38 (3H; t, J = 7), 1·95 (4H; m), 3·64 (2H; broad), 3·92 (6H; s), 4·34 (2H; q, J = 7), 4·44 (2H; broad), 7·37 (2H; s); Mass: M^+.

4-(N-*Nitroguanidino*)-1-*butanol* (XII)

N-Nitro-S-methylisothiourea (2·1 g)[5] was added to an ethanolic soln of XI (1·4 g in 2·5 ml). The reaction mixture was allowed to stand at room temp until the evolution of methylmercatan ceased (ordinarily a few hr at r.t.). The resulting crystalline mass was washed with EtOH to give 2·5 g (90%) of XII, m.p. 109·5–111° (dec) and recrystallized from MeOH-benzene, m.p. 114–115° (dec). (Found: C, 34·07; H, 7·10; N,

32·00: $C_5H_{12}O_3N_4$ requires: C, 34·08; H, 6·86; N, 31·80%); IR: ν^{KBr} 3475, 3390, 3250, 2940–2860, 1645, 1600, 1533 cm^{-1}; UV: λ_{max}^{MeOH} 222 (6,300), 271 mμ (ε = 18.300); NMR(DMSO-d_6-D_2O): 1·50 (4H; m), 3·20 (2H; broad), 3·45 (2H; broad).

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