Synthesis and X-ray crystallographic analysis of some 1,6-dihydro-1,2,4,5-tetrazines

Wei-Xiao Hu* and Feng Xu

College of Pharmaceutical Science, Zhejiang University of Technology Hangzhou 310032, P.R. China

Eight 1,6-dihydro-1,2,4,5-tetrazines (**2a–2h**) were synthesised by reaction of sodium borohydride with 3,6-substituted-1,2,4,5-tetrazines. The structure of **2c** was confirmed by single-crystal X-ray diffraction. The central six-membered ring of **2c** has an obvious unsymmetrical boat conformation. It could be considered that the molecule has a homoaromaticity.

Keywords: 1,6-dihydro-s-tetrazine, crystal structure, homoaromaticity, asymmetry, sodium borohydride

There are several reviews indicating that compounds containing the 1,2,4,5-tetrazine skeleton can be used as pharmaceuticals.[1,2] For example, 3-amino-6-aryl-1,2,4,5-tetrazines showed modest antimalarial activity and some hexahydro-s-tetrazines have been proved to have useful analgesic and antiflammatory activity. For a series of tetrahydro-s-tetrazines antibacterial and antifungal activities have been evaluated.

Recently our research team found that 3,6-dialkyl-1,6-dihydro-1,2,4,5-tetrazine is an important intermediate for the synthesis of some s-tetrazine derivatives, which have good antitumour activity, especially 1,4-dihydro-s-tetrazine-1,4-dicarboxamides.[3-7] To further investigate how the substituents located at the 3,6-positions of the 1,6-dihydro-s-tetrazine ring influence antitumour activity, eight 1,6-dihydro-1,2,4,5-tetrazines including seven new compounds have been synthesised. The chemical structures of and synthetic routes to the target compounds are shown in Fig.1.

To prepare **1a–f**, according to Lang *et al.*'s method,[8] the substituted ethyl benzimidate hydrochloride and acetamidine hydrochloride were reacted with hydrazine hydrate to form dihydro–s–tetrazines which were then oxidised by sodium nitrite and acetic acid. When preparing **1g–h**, according to Abdel–Rahman *et al.*'s method,[9] the substituted benzylnitriles were reacted with hydrazine hydrate, to form dihydro–s–tetrazines and then oxidised by sodium nitrite and acetic acid. Finally, **2a–h** including seven new compounds were prepared by using sodium borohydride as reductant according to Potman's method.[10] The results are summarised in Table 1.

When **1a** was reduced, there could be two isomers. One is **2a**, the other is **2a'**. In the observed NMR, it was found that the proton signal for CH_3 was a doublet coupled with a methine quartet in agreement with structure **2a**. The isomer **2a'** in which the proton is located at the carbon which is connected to a phenyl group could not be detected. In **2b–h**, the situation was the same.

Furthermore, when reducing 3,6-di(*p*-methylphenyl)-1,2,4,5-tetrazine and 3,6-diphenyl-1,2,4,5-tetrazine with sodium borohydride, 3,6-diaryl-1,6-dihydro-1,2,4,5-tetrazines were not obtained. Conversely, two yellow solids were

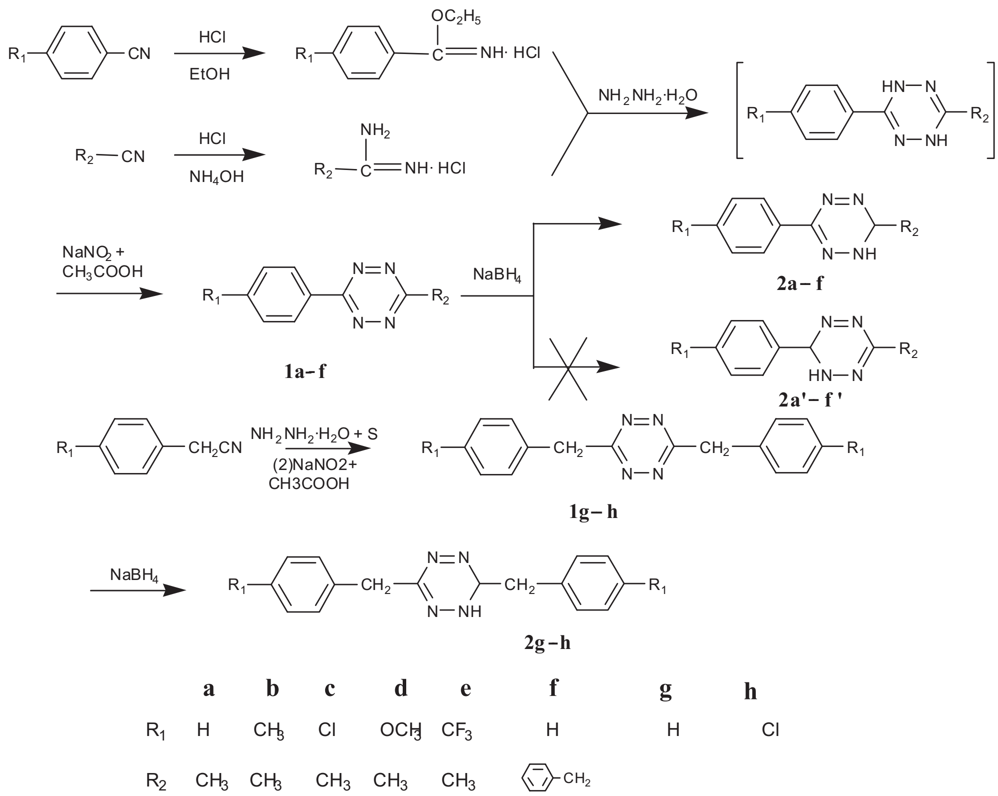
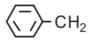


Fig.1 The synthetic route to the target compounds and their chemical structures.

* Correspondent. E-mail: huyang@mail.hz.zj.cn

Table 1 The preparation of **2a–h**

Entry	R_1	R_2	M.p./°C(Lit.)	Yield/%
2a	H	CH_3	107–108 (106.5–108[10])	47.1
2b*	CH_3	CH_3	116–118	40.6
2c*	Cl	CH_3	120–121.5	30.1
2d*	OCH_3	CH_3	105–107	35.4
2e*	CF_3	CH_3	110–112	31.0
2f*	H		90–92	22.5
2g*	H		96–98	17.8
2h*	Cl		98–99	21.4

*New coupounds.

obtained and identified as *N,N'*-bis-(4-methylbenzylidene)-hydrazine and *N,N'*-dibenzylidene-hydrazine.

In conclusion, we have found that the 1,6-dihydro-s-tetrazines can be synthesised only when the 6-position is occupied by an alkyl group. If the 3,6-substituents are aryl groups, the target products could not be obtained and *N,N*-benzylidene-hydrazine derivatives were obtained.

In addition to IR, NMR, MS and elemental analysis, structures were confirmed by X-ray crystallography of compound **2c**. (Fig. 2). The crystal data of **2c** are summarised in Table 2. The molecular structure shows that the *p*-chlorophenyl groups and methyl group are located at the 3-position and the 6–position of the s-tetrazine ring respectively and that two hydrogen atoms are at the 1,6-positions. The bond lengths of C_6–N_1 and C_6–N_5 are 1.431(2)Å and 1.472(2)Å respectively, which corresponds to C–N single bonds; the bond length of C_3=N_2 is 1.323(2)Å, which corresponds to a C=N double bond; the bond length of N_1–N_2 is 1.3134(19)Å, which corresponds to an N–N single bond; and the bond length of N_4=N_5 is 1.2605(18)Å, which corresponds to an N=N double bond. Therefore it is confirmed that **2c** is a 1,6-dihydro-s-tetrazine derivative. The bond length of C_3–N_4 is 1.388(2)Å, which is shorter than a C–N single bond (1.43 Å) but longer than that of C=N (1.32 Å).

In the central ring, the atoms N_1,N_2,N_4 and N_5 are coplanar, while atoms C_3 and C_6 deviate from the plane by 0.320(2) and 0.662(3)Å, respectively. The planes through N_2–C_3–N_4 and through N_1–C_6–N_5 make dihedral angles of 27.2° and 47.5°, respectively, with the plane through N_1–N_2–N_4–N_5. In fact, it forms an unsymmetric boat conformation. N_1, which carries the H_1, is almost SP^2 hybridised as the angles around it add up to 358.7(2)°. According to the similarity to 3-phenyl-6-methyl-1,6-dihydro-1,2,4,5-tetrazine[10] and 3-phenyl-6-ethyl-1,6-dihydro-1,2,4,5-tetrazine[11] suggests that the molecule can be considered as having homoaromaticity.

In addition, every molecule is involved in two hydrogen–bonding interactions, which contribute to the formation of the crystal structure. The hydrogen-bond geometry is summarised in Table 3.

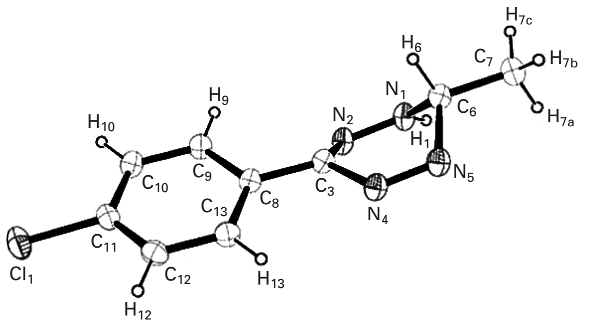


Fig. 2 Molecular structure of **2c**, shown with 30% probability displacement ellipsoids.

Experimental

Compounds **1a–f** were synthesised by a literature method[8] and compounds **1g–h** were synthesised by another literature method.[9] Solvents and reagents were commercially available and used without further purification.

X-ray single diffraction was carried out with an Enraf-Nonius CAD-4 diffractometer by the Analysis centre of Fu-Dan University. Data were collected and refined by CAD-4 EXPRESS. Program(s) used to solve and refine the structure were SHELXS97. Molecular graphics were solved by ORTEX. The software used to prepare material for publication was SHELXL97.

Melting points were measured on an XRC-1 apparatus and are uncorrected. IR spectra were recorded from KBr discs on a Nicolex FI-IR-170 instrument. ^{1}H NMR spectra were run on a Bruker AC400(400MHZ) spectrometer using TMS as internal standard and $CDCl_3$ as the solvent. Mass spectra were obtained on a HP5989A spectrometer at an ionising voltage of 70ev by electron impact. Elemental analyses were performed on a ThermoFinnigan Flash EA1112 instrument. For the ^{1}H NMR of AA'XX' systems $J^* = J_{23} + J_{25}$.

Table 2 The crystal data of **2c**

Empirical formula	$C_9 H_9 N_4 Cl$	
Formula weight	208.65	
Temperature	293(2) K	
Wavelength	0.71073Å	
Crystal system, space group	Monoclinic, P 21/c	
Unit cell dimensions	*a* = 9.948(3)Å	alpha = 90.000 deg.
	b = 8.833(3)Å	beta = 102.682(4) deg.
	c = 11.420(3)Å	gamma = 90.000 deg.
Volume	979(5)Å^3	
Z, Calculated density	4, 1.416 Mg/m^3	
Absorption coefficient	0.354 mm^{-1}	
F(000)	432	
Crystal size	0.20 × 0.15 × 0.10 mm	
Theta range for data collection	0.98 to 27.17 deg.	
Max. and min. transmission	0.9326 and 0.9655	

Table 3 Hydrogen–bond geometry (Å,°)

D–H•A	D–H	H•A	D•A	D–H•A
N_1–H_1•N_4^{i}	0.900(14)	2.802(15)	3.658(2)	159.3(15)
N_1–H_1•N_5^{i}	0.900(14)	2.025(15)	2.922(2)	174.6(16)
C_7–H_7C•N_4^{ii}	0.96	2.70	3.594(3)	156
C_{10}–H_{10}•N_2^{iii}	0.93	2.80	3.671(2)	156

Symmetry codes: (i) –*x*; *y* + 1/2; –*z* + 1/2; (ii) *x*; –*y* + 1/2; *z*–1/2; (iii)–*x* + 1; –*y* + 1; –*z* + 1.

3-Phenyl-6-methyl-1,6-dihydro-1,2,4,5-tetrazine (**2a**): **1a** (3-phenyl-6-methyl-1,2,4,5-tetrazine) (1.7 g, 10 mmol), ethanol (40 ml) and chloroform (20 ml) were mixed and cooled to –10°C. The sodium borohydride (380 mg, 10 mmol) and 95% ethanol (40 ml) were added dropwise with stirring. After the mixture was stirred for 15 min, some solid sodium borohydride (20 mg) was added in order to complete the reaction. The water (300 ml) and ammonium chloride (5 g) were added. After extraction of the water layer with chloroform, drying of the extract over anhydrous magnesium sulfate, and evaporation of the chloroform, crude 1,6-dihydro-1,2,4,5-tetrazine was obtained and purified by recrystallisation from ether-pentane to give about 0.7 g product as yellow crystals. Yield 47%. M.p. 107–108°C (Lit[10] 106.5–108°C). IR(KBr,cm^{-1}) 3444 s(N–H), 2900 m(C–H), 1650 m(C=N), 1395 s(ring). ^{1}H NMR ($CDCl_3$) δ ppm: 7.95 (d,2H, *J* = 9.6 Hz), 7.39–7.46 (m,2H,ArH), 7.38 (t,1H, *J* = 7.2 Hz), 2.42 (q,1H, *J* = 6.0 Hz), 2.04 (d,3H, *J* = 6.2 Hz). MS. (*m/z*, %). 146 (M–28,2.58), 131 (3.52), 104 (100), 91 (1.30), 77 (19.83), 42 (9.49).

3-(p-Methylphenyl)-6-methyl-1,6-dihydro-1,2,4,5-tetrazine (**2b**): Following the method used for **2a** with **1b** (0.4 g, 2.1 mmol) in 8 ml of ethanol, 4 ml of chloroform and 76 mg (2 mmol) of sodium borohydride in 8 ml of 95% ethanol and purified by preparative thin-layer chromatography over silica gel PF_{254} (2 mm) (petroleum ether : dichloromethane = 4 : 6) to give 160 mg product (41%) of **2b** as yellow crystals. M.p. 116–118°C. IR(KBr, cm^{-1}) 3440 s(N–H), 2936 m(C–H), 1635 m(C=N),1397 s(ring). ^{1}H NMR ($CDCl_3$) δ ppm: 7.83 (m,2H, *J** = 7.8 Hz), 7.25 (m,2H, *J** = 7.8 Hz), 2.40 (q,1H, *J* = 5.6), 2.39 (s,3H), 2.04 (d,3H, *J* = 6.0 Hz) MS. (*m/z*, %). 160 (M–28, 2.31), 145 (3.48), 118 (100), 91 (14.70), 77 (0.91), 42 (5.10). Anal. Calcd. for $C_{10}H_{12}N_4$ (188.23): C, 63.81; H, 6.43; N, 29.77. Found: C, 63.8; H, 6.4; N, 29.45.

3-(p-Chlorophenyl)-6-methyl-1,6-dihydro-1,2,4,5-tetrazine (**2c**): Following the method used for **2a** with **1c** (0.2 g, 1.0 mmol) in 4 ml of ethanol, 2 ml of chloroform and 38 mg (1 mmol) of sodium borohydride in 4 ml of 95% ethanol and purified by preparative thin-layer chromatography over silica gel PF_{254} (2 mm) (dichloromethane) to give 60 mg product (30%) **2c** as yellow crystals. M.p. 120–122°C. IR(KBr, cm^{-1}) 3441 s(N–H), 2938 m(C–H), 1634 m(C=N), 1394 s(ring), 711 m(C–Cl) ^{1}H NMR ($CDCl_3$) δ ppm: 7.88 (m,2H, *J** = 8.4 Hz), 7.25 (m,2H, *J** = 8.4 Hz), 2.40 (q,1H, *J* = 6.0 Hz), 2.04 (d,3H, *J* = 6.0 Hz). MS. (*m/z*, %). 180 (M–28,4.42), 165 (4.55), 138 (100), 111 (10.02), 102 (18.29), 75 (9.42), 42 (9.93) Anal. Calcd. for $C_9H_9ClN_4$ (208.65): C, 51.81; H, 4.35; N, 26.85.Found: C, 52.0; H, 4.4; N, 27.1.

CCDC 608819 contains the supplementary crystallographic data for **2c**. They can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request.cif.

3-(p-Methoxyphenyl)-6-methyl-1,6-dihydro-1,2,4,5-tetrazine (**2d**): Following the method used for **2a** with **1d** (0.3 g, 1.5 mmol) in 6 ml of ethanol, 3 ml of chloroform and 52 mg (1.5 mmol) of sodium borohydride in 6 ml of 95% ethanol and purified by preparative thin–layer chromatography over silica gel PF_{254} (2 mm) (dichloromethane) to give 107 mg product (35%) of **2d** as yellow crystals. M.p. 105–107°C. IR(KBr, cm^{-1}) 3457 s(N–H), 2934 m(C–H), 1611 m(C=N), 1392 s(ring). ^{1}H NMR ($CDCl_3$) δ ppm: 7.87 (m,2H, *J** = 9.2 Hz), 6.96 (m,2H, *J** = 9.2 Hz), 3.84 (s,3H), 2.32 (q,1H, *J* = 6.0 Hz), 2.04 (d,3H, *J* = 6.0 Hz). MS. (*m/z*, %). 176 (M–28,13.56), 161 (8.64), 133 (100), 119 (6.78), 103 (15.82), 90 (16.32), 77 (5.71), 42 (3.82). Anal. Calcd. for $C_{10}H_{12}N_4O$ (204.23): C, 58.81; H, 5.92; N, 27.43. Found: C, 58.95; H, 5.6; N, 27.2.

3-(p-Trifluoromethylphenyl)-6-methyl-1,6-dihydro-1,2,4,5-tetrazine (**2e**): Following the method used for **2a** with **1e** (0.8 g, 3.3 mmol) in 15 ml of ethanol, 8 ml of chloroform and 129 mg (3.3 mmol) of sodium borohydride in 15 ml of 95% ethanol and purified by preparative thin-layer chromatography over silica gel PF_{254} (2 mm) (chloroform : petroleum ether = 8 : 2) to give 250 mg product (31%) of **2e** as yellow crystals. M.p. 110–112°C. IR(KBr, cm^{-1}) 3438 s(N–H), 2937 m(C–H), 1617 m(C=N), 1322 s(ring). ^{1}H NMR ($CDCl_3$) δ ppm: 8.04 (m,2H, *J** = 8.8 Hz), 7.68 (m,2H, *J** = 8.8 Hz), 2.44 (q,1H, *J* = 6.4 Hz), 2.04 (d,3H, *J* = 6.4 Hz). MS. (*m/z*, %). 214 (M–28,2.17), 199 (4.23), 172 (100), 145 (20.83), 121 (16.15), 95 (5.24), 75 (8.67), 42 (29.01). Anal. Calcd. for $C_{10}H_9F_3N_4$ (242.20): C, 49.59; H, 3.75; N, 23.13.Found: C,50.0; H, 3.7; N, 23.3.

3-Phenyl-6-benzyl-1,6-dihydro-1,2,4,5-tetrazine (**2f**): Following the method used for **2a** with **1f** (1.5 g, 6 mmol) in 24 ml of ethanol, 12 ml of chloroform and 228 mg (6 mmol) of sodium borohydride in 24 ml of 95% ethanol and purified by preparative thin–layer chromatography over silica gel PF_{254} (2 mm) (petroleum ether : dichloromethane = 4 : 6) to give 340 mg product (23%) of **2f** as yellow crystals. M.p. 90–92°C. IR(KBr, cm^{-1}) 3442 s(N–H), 2963 m(C–H), 1633 m(C=N), 1393 s(ring). ^{1}H NMR ($CDCl_3$) δ ppm: 7.94 (t,2H, *J* = 8.8 Hz), 7.36–7.47 (m,8H,ArH), 3.73 (d,2H, *J* = 9.0 Hz), 2.74 (t,1H, *J* = 9.5 Hz). MS. (*m/z*, %). 222 (M–28,61.40), 194 (22.84), 178 (2.38), 145 (8.06), 117 (57.74), 91 (100), 77 (81.46), 51 (22.99). Anal. Calcd. for $C_{15}H_{14}N_4$ (250.30): C, 71.98; H, 5.64; N, 22.38. Found: C, 71.7; H, 5.7; N, 22.2.

3,6-Dibenzyl-1,6-dihydro-1,2,4,5-tetrazine (**2g**): Following the method used for **2a** with **1g** (2.8 g, 10.6 mmol) in 40 ml of ethanol, 20 ml of chloroform and 380 mg (10 mmol) of sodium borohydride in 40 ml of 95% ethanol and purified by preparative thin-layer chromatography over silica gel PF_{254} (2 mm) two times (petroleum ether : dichloromethane = 5 : 5, cyclohexane) to give 500 mg product (18%) of **2g** as yellow crystals. M.p. 96–98°C. IR(KBr, cm^{-1}) 3435 s(N–H), 2940 m(C–H), 1633 m(C=N), 1386 s(ring). ^{1}H NMR ($CDCl_3$) ppm: 7.26–7.32 (m,10H,ArH), 4.16 (s,2H), 3.07 (d,2H, *J* = 6.0 Hz), 2.50 (t,1H, *J* = 5.6 Hz). MS. (*m/z*, %). 236 (M–28,10.29), 145 (100), 118 (43.24), 91 (99.63), 77 (7.50), 65 (23.43). Anal. Calcd. for $C_{16}H_{16}N_4$ (264.33): C, 72.70; H, 6.10; N, 21.20.Found: C, 72.4; H, 6.2; N, 21.2.

3,6-Di(p-chlorobenzyl)-1,6-dihydro-1,2,4,5-tetrazine (**2h**): Following the method used for **2a** with **1h** (1.3 g, 4.3 mmol) in 16 ml of ethanol, 8 ml of chloroform and 156 mg (10 mmol) of sodium borohydride in 16 ml of 95% ethanol and purified by preparative thin–layer chromatography over silica gel PF_{254} (2 mm) twice (petroleum ether : dichloromethane = 5 : 5, cyclohexane) to give 280 mg product (21.4%) of **2h** as yellow crystals. M.p. 98–99°C. IR(KBr,cm^{-1}) 3444 s(N–H), 2927 m(C–H), 1635 m(C=N), 1489 s(ring). ^{1}H NMR ($CDCl_3$) δ ppm: 7.15–7.32 (m,8H,ArH), 4.10 (s,2H), 3.55 (d, 2H, *J* = 6.0 Hz), 2.41 (t,1H, *J* = 6.0 Hz). MS. (*m/z*, %). 304 (M–28,1.92), 178 (100), 151 (11.75), 125 (35.55), 117 (19.52), 77 (3.97), 63 (5.16). Anal. Calcd. For $C_{16}H_{14}Cl_2N_4$ (333.22): C, 57.67; H, 4.23; N, 16.81.Found: C, 57.3; H, 4.2; N, 16.5.

We are indebted to the National Natural Science Foundation of China (grant No. 20272053) for financial support.

Received 11 July 2006; accepted 17 October 2006
Paper 06/4074

References

1 H. Neunhoeffer, *Comprehensive Heterocyclic Chemistry*, A. McKillop and A.J. Boulton, (eds), Pergamon, Oxfords, 1984, Vol. 3, pp.531–572.
2 J. Sauer, *Comprehensive Heterocyclic Chemistry II*, A.J. Boulton, (ed.), Elsevier, Oxford, 1996, Vol. 6, pp 901–953.
3 G.W. Rao and W.X. Hu, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 3174.
4 W.X. Hu, H.B. Shi, Q. Yuan and Y.Q. Sun, *J. Chem. Res.*, 2005, 91.
5 W.X. Hu, G.W. Rao and Y.Q. Sun, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1177.
6 W.X. Hu, Y.Q. Sun and Q. Yuan, *Chem. J. Chin. Univ.*, 2002, **23**, 1887.
7 Z.B. Cai, M. Zhou, W.X. Hu and Z.Y. Yang, *Acta Pharmaceut., Sin.*, 2000, **35**, 793.
8 S.A. Lang, Jr., B.D. Johnson, E. Cohen, A.E. Sloboda and E. Greenblatt, *J. Med. Chem*. 1976, **19**, 1404.
9 M.O. Abdel-Rahman, M.A. Kira and M.N. Talba, *Tetraedron Lett.*, 1968, **35**, 3871.
10 A. Counotte-Potman, H.C. van der Plas and B. van Veldhuizen, *J. Org. Chem.*, 1981, **46**, 2138.
11 C.H. Stam, A.D. Counotte-Potman and H.C. van der Plas, *J. Org. Chem.*, 1982, **47**, 2856.