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NORDITERPENOID AND DITERPENOID ALKALOIDS FROM THE ROOTS OF *ACONITUM NASUTUM* FISCH. EX REICHB.

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Abstract - Investigation of the alkaloidal constituents of the roots of *Aconitum nasutum* led to the isolation and identification of a new diterpenoid alkaloid designated as *trabzonine* (**1**). Three known norditerpenoid alkaloids, lappaconitine (**2**), lycoctonine (**3**) and gigactonine (**4**), and two known diterpenoid alkaloids pseudokobusine (**5**) and septatisine (**6**) were also isolated. The structures of **1** and **5** were established by detailed analyses of ^{1}H and ^{13}C NMR data. The structure and absolute stereochemistry of pseudokobusine (**5**) was determined by an X-Ray crystal structure determination.

INTRODUCTION

Earlier investigations of the roots of *Aconitum nasutum* Fisch. ex Reichb. resulted in the isolation of the two norditerpenoid alkaloids: aconitine and acetylaconosine.[1] From the aerial parts the following eleven alkaloids were isolated: aconosine,[2] cammaconine, columbianine, isotalatizidine, karakoline, talatisamine,[3] 14-acetyltalatisamine, anthranoyllycoctonine, columbidine, 3-hydroxytalatisamine, lycoctonine[4] and aconasutine[5].

RESULTS AND DISCUSSION

From the roots of *A. nasutum* collected at an altitude of 700 m in Trabzon-Sürmene, Turkey, we have isolated a novel diterpenoid alkaloid (**1**) designated as *trabzonine*, mp 123-126^{o}C; $[\alpha]_{D}^{25}$ -5^{o} (c 1.2, MeOH) . The molecular formula, $C_{22}H_{33}NO_{3}$ (ESI, MW, M^{+}+1, m/z

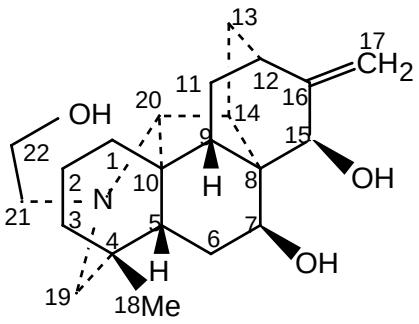
360) was derived for the alkaloid by HRFABMS (M^++1, m/z 360.25360; calcd. 360.25388) and confirmed by the ^{13}C NMR spectral and DEPT data. The ^{1}H NMR spectra did not show signals for methoxyl, *N*-alkyl or acetoxyl groups which are characteristic of norditerpenoid alkaloids, suggesting that trabzonine is a diterpenoid alkaloid. The ^{13}C NMR spectrum showed 22 signals, of which four are singlets, seven doublets, ten triplets, and one quartet for 22 carbon atoms of the molecule appearing at ppm: 157.1 (s), 104.3 (t), 76.4 (d), 69.6 (d), 68.1 (d), 58.9 (t), 58.4 (t), 56.9(t), 49.9 (s), 44.9 (d), 44.6 (s), 44.3 (d), 44.3 (d), 41.6 (t), 35.9 (t), 34.5 (d), 34.0 (s), 33.0 (t), 32.0 (t), 29.2 (t), 28.2 (q), and 20.6 (t). The 22 carbon atoms of trabzonine and the methylene signals at 58.4 and 58.9 ppm in the ^{13}C NMR spectrum indicated the presence of an -*N*-CH_2CH_2O- functionality in the molecule. The signals at 157.1 (s, C-16) and 104.3 (t, C-17) ppm suggested that the alkaloid is of the atisane-type and these signals should be assigned to the exocyclic methylene group.[6] Two broad singlets at δ 4.87 and 4.98 in the ^{1}H NMR spectrum supported this suggestion. The ^{13}C NMR methine signals at 69.6 and 68.1 ppm supported by the upfield ^{1}H NMR signals at δ 4.24 and 4.52, respectively, indicated that two of the carbons of the molecule are attached to oxygen functions and in all probability, hydroxyl groups.

Three different scalar-coupled spin systems were delineated by the homonuclear ^{1}H COSY NMR spectrum. (Table 1): [H-1-H-2-H-3α-H-3β], [H-5-H-6α-H-6β-H7], [H-9-H-11α-H-11Hβ-H-12-H-13-H-14-H-20]. The carbon and the one-bond ^{1}H-^{13}C coupled protons were assigned using DEPT and COSY (HETCOR) spectra. The C-17 exocyclic methylene protons (δ 4.87 and 4.98) showed significant coupling to a methine proton (δH 4.52 br s; δC 67.9) in the COSY spectrum attributed to a carbon bearing a hydroxyl group assigned to H-15. The exocyclic methylene protons were distinguished since one of them at δ 4.87 showed a strong nOe to H-12 only (δ H 2.26 ; δ C 34.5). Therefore the protons at δ 4.98 and 4.87 were assigned to H-17a and H-17b, respectively. The methine proton (δH 4.24, dd J=8.7, 7.8 Hz; δC 69.6) showed a COSY correlation with H-6a and H-6b (δH 2.16, 1.92 ; δC 33.0) and H-6a and H-6b correlated with (δH 1.18; δC 44.9) which is assigned to the H-5 proton. The third oxygen which is a hydroxyl group is attached to C-22 (δC 58.9; δH 3.71, 3.54). On the basis of these data, the structure (**1**) was assigned to trabzonine.

Norditerpenoid alkaloids isolated from the roots of this plant are: lappaconitine (**2**), lycoctonine (**3**) and gigactonine (**4**). These were identified by comparison of their ^{1}H and ^{13}C NMR spectra with those of authentic samples.[7] A known diterpenoid alkaloid obtained from this plant is shown to be identical with septatisine (**6**) isolated earlier from *Aconitum septentrionale*, Koelle.[6] As trabzonine (**1**) and septatisine (**6**) are closely similar, the ^{13}C and ^{1}H NMR data have been given table 1.[7]

Another crystalline alkaloid isolated from the roots indicated a molecular formula $C_{20}H_{27}NO_3$ (HRFABMS: M^++1, m/z 330.20692; calcd. 330.1980). As the ^{13}C NMR spectrum of this compound could not be compared with the spectra of known alkaloids and because of the

paucity of material, we undertook the X-Ray crystal structure and a detailed NMR investigation. The alkaloid proved to be pseudokobusine (**5**) which has been isolated previously from *Aconitum yesoensis* and *A. yesoense* var*. macroyesoense*. [8,9] Since kobusine (**7**) has been correlated with pseudokobusine and the absolute stereochemistry of kobusine has been established,[8,10] pseudokobusine has the absolute stereochemistry: 4*R*, 5*R*, 6*R*, 8*R*, 9*S*, 10*S*, 11*R*, 12*R*, 14*S*, 15*S*, 20*R*. An ORTEP diagram of pseudokobusine is shown in Figure 1. Accurate assignments for the proton and carbon atoms of pseudokobusine (**5**) have been made (Tables 2 and 3) on the basis of heteronuclear ^{1}H decoupled ^{13}C, DEPT, 2D ^{1}H COSY, ROESY, HMQC, and HMBC NMR spectral studies.



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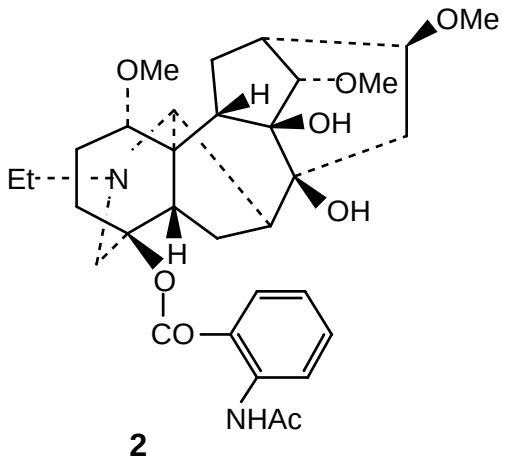

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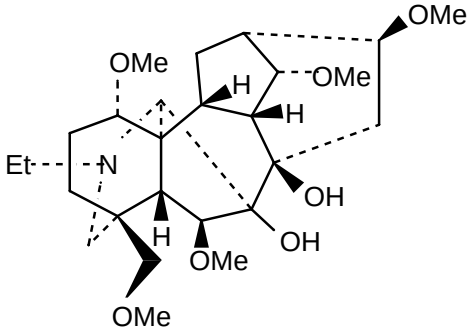

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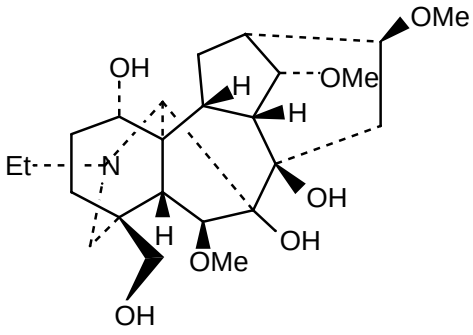

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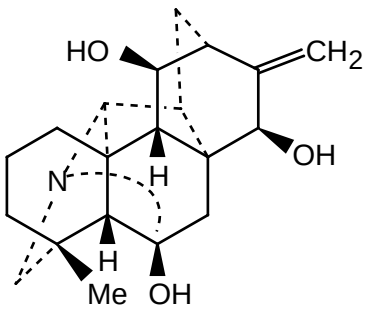

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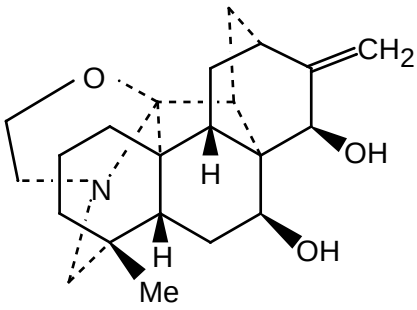

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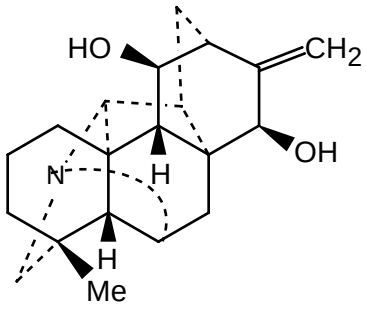

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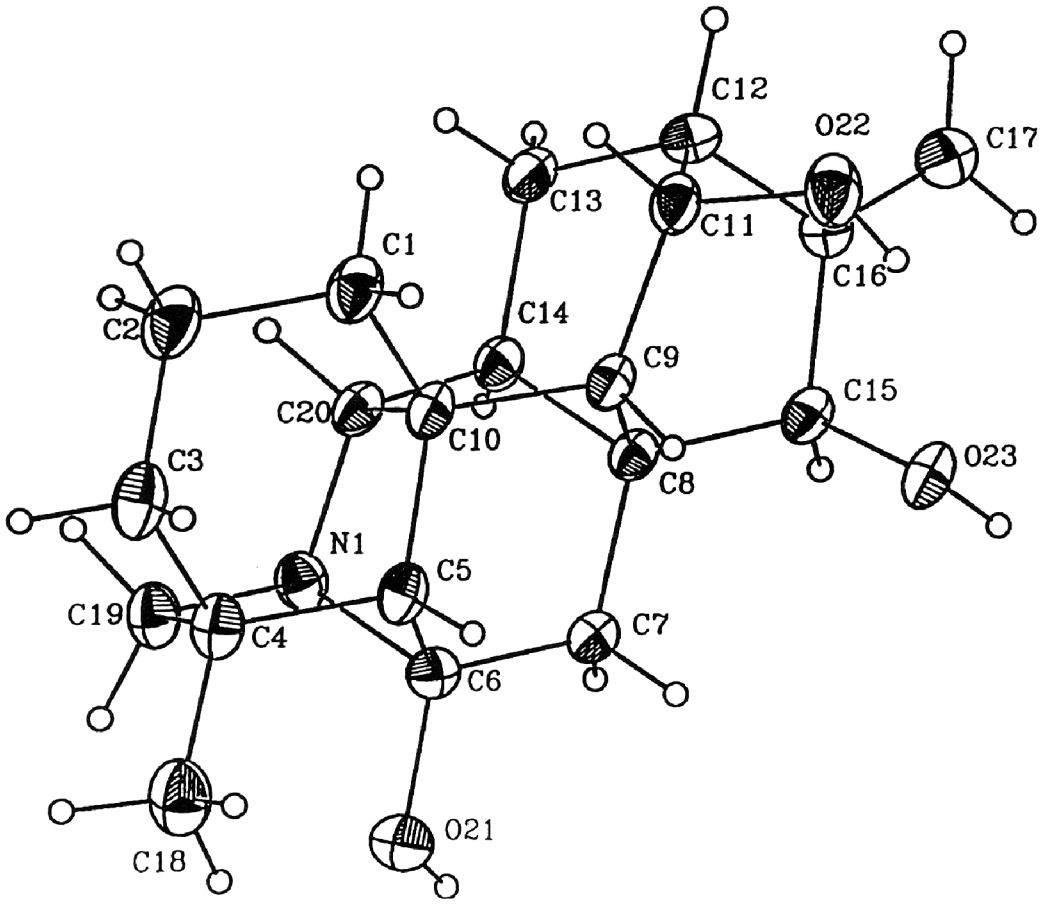

Figure 1. ORTEP Drawing of Pseudokobusine (**5**)

Table 1. NMR data for Trabzonine (**1**) and Septatisine (**6**) in $CDCl_3$

	13C (M)			1H δ				
Position	**1**	**6**		**1**	**6**	COSY	ROESY	HMBC (C→H)
1	32.0	30.2	t	1.84	2.20	2	2	–
					0.90_{β}	1, 3_{α}, 3_{β}		
2	20.6	19.6	t	1.52	1.55_{α}	2	1.3	–
					1.45_{β}	–	–	–
3	41.6	41.3	t	1.33_{α}	1.25_{α}	2	18	H-19_a, H-19_b
				1.30_{β}	1.11_{β}	–	–	–
4	34.0	34.4	s	–	–	–	–	H-18, H-19
5	44.9	46.6	d	1.18	1.25	6_{α}, 6_{β}, 20	6_{α}, 6_{β}	H-6_{α}, H-6_{β}, H-7, H-18,
6	33.0	23.3	t	2.16_{α}	2.28_{α}	5, 7	5, 7, 18	–
				1.93_{β}	2.10_{β}	–	–	–
7	69.6	70.0	d	4.24	4.21	6_{α}, 6_{β}	6_{α}, 6_{β}, 20	–
8	49.9	50.0	s	–	–	–	–	H-6_{α}, H-6_{β}, H-20
9	44.3	44.0	d	1.55	1.45	11_{α}, 11_{β}	14	H-12
10	44.6	47.1	s	–	–	–	–	H-6_{α}, H-6_{β}, H-20
11	29.2	29.2	t	1.92_{α}	2.10_{β}	9, 12	13	–
				1.56_{β}	1.55_{β}	–	–	–
12	34.5	34.5	d	2.26	2.20	11_{α}, 11_{β}, 13_{α}, 13_{β}	13, 14, 17	H-17_a, H-17_b
13	35.9	27.4	t	1.62_{α}	1.98_{α}	12, 14	11, 12	–
				1.49_{β}	1.36_{β}	–	–	–
14	44.3	49.6	d	2.11	2.01	13_{α}, 13_{β}, 20	9, 12, 15, 20	H-12, H-15
15	68.1	68.7	d	4.52	4.49	17_a, 17_b	14, 17_a, 17_b	H-17_a, H-17_b
16	157.1	157.9	s	–	–	–	–	H-17_a, H-17_b
17	104.3	103.8	t	4.98_a	4.83_a	15	12, 15	–
				4.87_b	4.94_b	–	–	–
18	28.2	28.6	q	0.97	1.00	–	3, 6_{α}, 6_{β}, 19	H-19
19	56.9	57.3	t	2.73_{α}	2.35_{α}	19_{α}, 19_{β}	18, 20	H-18, H-21
				2.13_{β}	2.38_{β}	–	–	–
20	76.4 d	104.6 s	–	2.35	–	5, 14	6, 14, 19, 21	H-5, H-19
21	58.4	51.5	t	2.86_a	2.81_a	22_a, 22_b	20, 22_a, 22_b	H-20
				2.51_b	3.03_b	–	–	–
22	58.9	61.7	t	3.71_a	3.78_a	21_a, 21_b	19, 21_a, 21_b	–
				3.54_b	3.56_b			

Table 2. ^{13}C and ^{1}H NMR Chemical Shift Assignments of Pseudokobusine (**5**) in $CDCl_3$

Carbon Position	δ C		Proton	δ H	δ H (J=Hz)
1	27.4	t	H-1a	1.60	1H, m
			H-1b	1.36	1H, m
2	19.2	t	H-2a	1.57	1H, m
			H-2b	1.39	1H, m
3	35.5	t	H-3a	1.43	1H, m
			H-3b	1.30	1H, m
4	37.6	s	-		
5	61.2	d	H-5	1.44	1H, s
6	97.8	s	-		
7	40.2	t	H-7a	2.30	1H, m
			H-7b	1.54	1H, m
8	46.8	s	-		
9	54.1	d	H-9	1.62	1H, br s
10	49.8	s	-		
11	67.5	d	H-11	3.94	1H, d, J=4.84
12	34.5	d	H-12	2.41	1H, m, J= < 2 Hz
13	29.1	t	H-13a	1.72	1H, br s, w1/2 9.2 Hz
			H-13b	0.85	1H, d, J=9.2 Hz
14	40.7	d	H-14	1.72	1H, m
15	70.3	d	H-15	3.85	1H, br s
16	149.3	s	-		
17	114.9	t	H-17z	5.15	each 1H, br s
			H-17e	5.05	each 1H, br s
18	30.3	q	H-18	1.28	3H, s
19	60.0	t	H-19a	3.00	1H, Jab=11.9
			H-19b	2.26	1H, Jab =11.9
20	73.4	d	H-20	2.41	1H, s

Table 3. Summary of COSY, ROESY and HMBC Correlation Data of Pseudokobusine (**5**)

Obs. ^{1}H	COSY	ROESY	HMBC (H→C)
1a	H-2a,H- 2b	H-2a, H-2b,H-13b, H-20	C-5, C-20
1b	H-2a,H- 2b	H-2a, H-2b,H-13b, H-20	C-5, C-20
2a	H-1a, H-1b H-3a, H-3b	H-1a, H-1b	C-3, C-4
2b	H-1a, H-1b H-3a, H-3b	H-1a, H-1b	C-3, C-4
3a	H-2a, H- 2b	H-19b	C-4, C-18
3b	H-2a, H- 2b		C-4, C-18
5	-	H-7, H-18	C-7, C-9, C-19, C-20
7	H7a, H-7b	H-5	C-6, C-8, C-14
9	H-11	H-11	C-8, C-10, C-14, C-15, C-20
11	H-9, H-12	H-9, H-12, H-13b	C-8, C-10, C-16, C-17
12	H-11, H-13a, H-13b	H-11, H-13a, H-13b, H-17e	C-13, C-14
13a	H-12, H-14	H-12, H-13b, H-15	C-12, C-14
13b	H-12, H-14	H-11, H-12, H-13a, H-14, H-20	C-12, C-14
14	-	H-13b, H-15	C-9, C-10, C-15
15	H17z, H-17e	H-14, H-17z, H-17e (ω)	C-8, C-9, C-14, C-16, C-17
17z	H-15	H-15	C-12, C-15, C-16
17e	H-15	H-12, H-15 (ω)	C-12, C-15, C-16
18	-	H-5, H-19	C-4, C-19
19a	H-19b	H-18, H-19b	C-3, C-4, C-20
19b	H-19a	H-2, H-3, H-19a	C-3, C-4, C-20
20	H-14	H-1b, H-2a, H-13b	C-5, C-6, C-8

EXPERIMENTAL

General Experimental Procedures: Melting points are corrected and were determined on a Thomas Koffler hot stage equipped with a microscope and a polarizer. Optical rotations were measured on Perkin-Elmer Model 141 polarimeter in $CHCl_3$. MS and HRMS were determined on a VG Zap Specinstrument and Autospecmass spectrometers. Chromatographic separations on a Chromatotron[11] were carried out on rotors coated with 1mm thick layer of Al_2O_3 60 PF-254, 365 (EM 1104.3) or SiO_2 60 HF (EM 7749); VLC[12] was carried out with Merck Al_2O_3 (EM 1085) and SiO_2 60 (EM 7736). Thin layer chromatograms were run using the solvent system toluene:acetone:methanol:NH_4OH, 49.5: 41.5:8:5.

All NMR data were acquired at 22^o C on a Varian Inova 500 spectrometer. ^1H and ^{13}C Chemical shifts at 22 ^{o} C were referenced to TMS , via the $CDCl_3$ resonance frequency at 7.27 and 77 ppm, respectively. The 2D ^1H phase sensitive COSY and clean TOCSY spectra were obtained using a spectral width of 4.0 kHz for both dimensions, while 2D ROESY experiments were acquired with a spectral width of 8.0 kHz. For 2D TOCSY experiments, a spin lock field of 8 kHz was used during spin lock time of 60 ms, which includes 2 ms trim pulses. ROESY spectra were recorded with a spin lock field of 1.8 kHz during the 200-ms mixing time. For 2D ^1H-^{13}C heteronuclear experiments, a spectral width of 20 khz was used in the ^{13}C dimension for HMQC, HMQC-clean-TOCSY and 30 Khz for HMBC. ^1H-^{13}C coupling constants of 150 Hz and 9 Hz were used in HMQC and HMBC experiments, respectively. Quadrature detection in the indirectly observed dimensions was obtained using TPPI (time proportional phase increment) method for all 2D experiments. Typically, the data were acquired with acquisition time of 200 ms, 8 scans for each of FID's in the homonuclear experiments, and 32 scans for each of 256 FIDs in the heteronuclear experiments and 16 scans for each of 128 FID's in the heteronuclear experiments. 1D (300.13 MHz) and ^{13}C (75.47 MHz) and selective INEPT nmr spectra were recorded on a Bruker AC-300 spectrometer. The ^{13}C chemical shift multiplicities were determined from DEPT spectra.

Plant material : The roots of *A. nasutum* were collected at an altitude of 700 m in Trabzon-Sürmene, Arpali village, Turkey in 1997 and identified by Dr. Mustafa Küçükislamoglu. A voucher specimen (no. 15) has been deposited in the University of Istanbul herbarium.

Isolation of the alkaloids : Dried and finely powdered roots (400g) were exhoustively extracted at room temperature with 95% EtoH (5x3L). Usual work up gave a crude alkaloidol mixture (12g). The mixture was first separated by VLC on basic Al_2O_3 with hexane-$CHCl_3$-MeOH mixtures. VLC fraction 13 (hexane-$CHCl_3$ 4:6)(730 mg) was chromatographed on a SiO_2 rotor with hexane-$CHCl_3$-MeOH mixtures to furnish lappaconintine (**2**, 49 mg). VLC fraction 27 ($CHCl_3$-MeOH 98:2)(582 mg) was chromatographed on a SiO_2 rotor with hexane-$CHCl_3$-MeOH mixtures to give lycoctonine (**3**, 160 mg). VLC fraction 29-30 ($CHCl_3$-MeOH 97:3) (420 mg) were chromatographed on a SiO_2 rotor with hexane-$CHCl_3$-MeOH mixtures to give gigactonine (**3**, 163 mg). VLC fraction 31 ($CHCl_3$-MeOH 96:4) (221 mg) was

chromatographed on a SiO_2 rotor with hexane-$CHCl_3$-MeOH mixtures to afford trabzonine (**1**, 9 mg) as crystalline cubes, mp 123-126°C. VLC fractions 32-36 ($CHCl_3$-MeOH 95:5, and 90:10) (942 mg) were chromatographed on a basic Al_2O_3 rotor with hexane-$CHCl_3$-MeOH mixtures to give septatisine (**6**, 10 mg) and pseudokobusine (**5**, 30 mg), mp 271-272 °C.

Table 4. Crystallographic Data of Pseudokobusine (**5**)

Formula:	$C_{20}\,H_{27}\,N\,O_3$
Mol. wt.:	329.43
Space Group:	monoclinic, P2(1)
α	8.0746(8) Å
β	11.4613(11) Å
c	9.1121(9) Å
β	90.338(2) °
Unit cell volume ³:	843.27(14) $Å^3$
D_{calc}.(gcm cube):	1.297 g cm^{-1}
Z:	2
Abso. Coeff.	0.086
Radiation used:	Mo *K* α
Cryst. dimensions	0.29x0.21x0.19
Crystals	Colorless
θ range	3-53 °
Unique data:	2045
Observed data:	1938
Criterion for obs,	>2Sigma(I)
Refined parameters	222
R factor:	0.0266
Weighted *R* factor	0.0681
Max residue density	0.117e $Å^3$
Min residue density	-0.105e $Å^3$

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