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Solvent Dependence of Absorption and Fluorescence Spectra of Piroxicam. A Possible Intramolecular Proton Transfer in the Excited State

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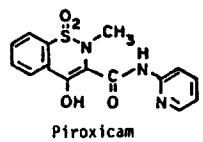
The spectral properties of piroxicam in different solvents are similar to those of its skeletal precursor, HMBDC. The maximum absorption and emission wavelengths strongly depend on the hydrogen bonding ability of the solvent, and it is shown that intramolecular hydrogen bonding between the –OH and the ortho carbonyl group of the parent benzothiazine ring plays an important role in the solvent-dependence of their spectroscopic properties. The fluorescence spectra in aprotic nonpolar solvent exhibit abnormally large Stokes-shifted (~9,000cm^{-1}) emission bands in contrast to the spectra in water. In ethanol, dual emission bands with two different fractional components of lifetimes have been observed. These results suggest that the abnormally red-shifted emission is attributed to the proton transferred form of an intramolecularly hydrogen-bonded closed conformer.

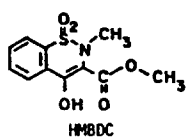
Introduction

Piroxicam (4-hydroxy-2-methyl-N-2-pyridine-1 ,2H-benzothiazine-3-carboxamide-1,1-dioxide), a non-steroidal and anti-inflammatory drug has been shown to cause cutaneous photosensitivity in some patients[1-4]. Even though its photosensitizing properties have not been understood, the photosensitization can be expected to occur with respect to red blood cells like many other photosensitizing drugs[5,6]. Thus the photosensitization may involve piroxicam in various microenvironmental conditions which depend on its incorporation into the red blood cells. In order to understand the photochemistry and the photophysics of piroxicam with respect to the photosensitization mechanism in biological molecules, it is of primary importance to investigate its electronic properties in various solvents such as nonpolar or polar solvents and/or hydrogen bonding solvents. Thus we have investigated the spectroscopic properties of piroxicam in various solvents.

It is the purpose of this paper to show that piroxicam displays absorption spectra and quite unusual fluorescence spectra both of which strongly depend upon the hydrogen bonding ability of the solvent at room temperature. The comparison with the solvent dependence of fluorescence properties obtained for a skeletal precursor of piroxicam, HMBDC (4-hydroxy-2-methyl-1,2-λ-benzothiazine-1,1-dioxide-3-methyl-carboxylate) infers to a possibility of the occurrence of an excited state intramolecular proton transfer from the hydroxyl group of the benzothiazine ring to the carbonyl portion of the amide group of the side chain. The latter plays an important role in the observation of an abnormally red-shifted fluorescence emission of piroxicam in nonpolar solvent.



Piroxicam



HMBDC

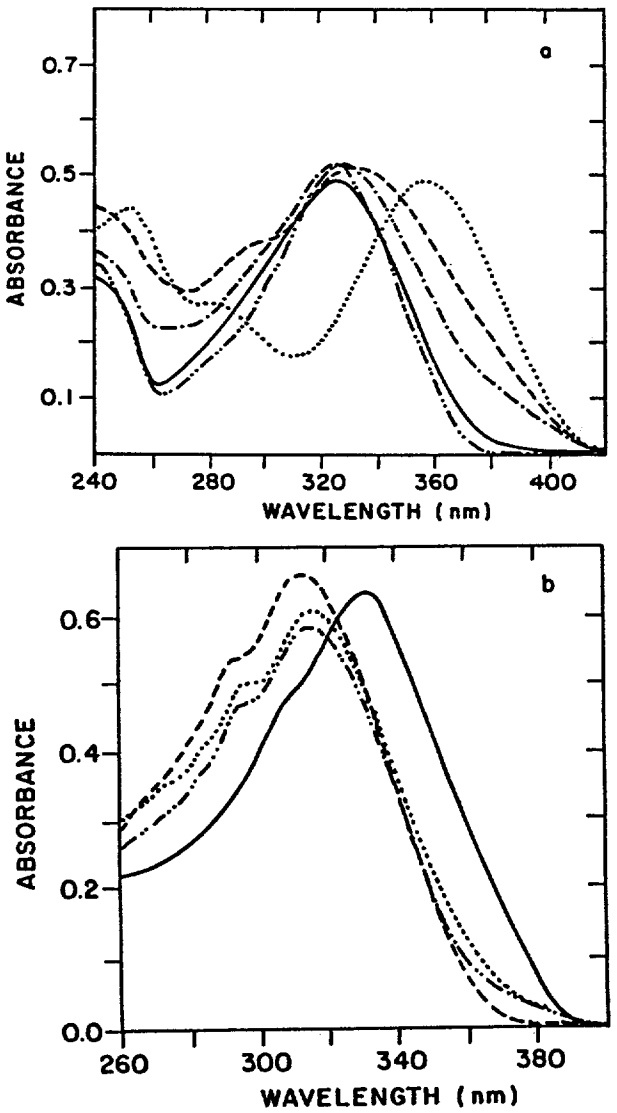


Figure 1. Absorption spectra of piroxicam and HMBDC in various solvents: (a) 4.0×10^{-5}M solution of piroxicam: (—··—) n-hexane; (— — —) dioxane; (—·—) butanol; (— — —) ethanol; (·····) H_2O. (b) 5.0×10^{-5}M solution of HMBDC. (— — —) n-hexane; (—··—) butanol; (····) methanol; (———) H_2O.

Experimental

Piroxicam was obtained from Yu Han Pharmaceutical Corporation in Korea and further purified by recrystallizing three times from methanol to produce bright white crystals. Its melting point was the same as the literature value (198-200 °C)[1,7]. HMBDC was synthesized by modifying the methods described elsewhere[8]. Coupling of N-acetyl saccharin with 2-equivalent sodium ethoxide in dimethyl formamide at 5 °C produced the sodium salt of 3-acetyl analog. By successive treatment of the sodium salt of 3-acetyl analog with 2-equivalent NaH and methyl iodide, the final product HMBDC was obtained. Further purification was performed by three successive recrystallization from ethanol. Its purity was checked by gas chromatography and the structural formulate were confirmed by NMR spectroscopic data: δ 3.3, δ 3.5 (NCH_3 and OCH_3), δ7.5 (C_6H_4), δ4.2 (exchangeable OH). Its melting point was 166-168 °C.

The organic solvents used for spectral measurements (*i.e.* methanol, ethanol, chloroform) were purchased from Merck Co, in the best spectroqualities available. Dioxane and n-hexane were purified by twice successive distillation, followed by refluxing over NaOH and dried with $CaCl_2$. The neat solvents did not show fluorescence under the practical conditions of spectral recc ing. Water was purified by triple distillation in the presence of $KMnO_4$.

Absorption spectra were measured on a Beckman UV-5260 spectrophotometer. The corrected fluorescence emission and excitation spectra were measured on SLM-AMINCO 4800 spectrofluorometer or Spex-Fluorolog II spectrofluorometer. When the fluorescence spectra were measured, a narrow excitation slit (< 4 nm) was used to minimize the photolysis of piroxicam and HMBDC. All spectral measurements were made at room temperature. Fluorescence quantum yields were determined by using quinine bisulfate (10^{-5} M in 1N H_2SO_4) as a standard, assuming a quantum yield of 0.55[9]. A quadratic correction for refractive index variation was applied. The concentration of sample solutions were of the order 10^{-5}-10^{-6} M. Fluorescence lifetimes were determined by using the time-correlated single-photon counting technique on the PRA 3000 system equipped with a N_2 (0.5 atm) arc lamp as described elsewhere[10]. The deconvolution was carried out with the iterative method employing a PRA software. The fit was judged on the basis of χ^2 reductions and the plot of the weighted residuals. In order to monitor emission wavelength, narrow bandwidth (10 nm) interference filters (Ditric Optics Inc.) were used.

Table 1. Solvent Effect on the Characteristics of the first Absorption Band of Piroxicam and HMBDC

Solvent	Piroxicam		HMBDC	
	λ_{max}, nm	ε, $cm^{-1}M^{-1}$	λ_{max}, nm	ε, $cm^{-1}M^{-1}$
Cyclohexane	326	1.3×10^4	—	1.3×10^4
Hexane	326	1.3×10^4	310	—
Dioxane	326	1.2×10^4	309	1.3×10^4
Buthanol	326	1.3×10^4	311	1.1×10^4
Methanol	336	1.3×10^4	311	1.2×10^4
Water	360	1.2×10^4	331	1.3×10^4

Results and Discussion

Absorption properties. The absorption spectra of benzothiazine derivatives, piroxicam and HMBDC were observed in different solvents and are shown in Figures **1a** and **1b**, respectively. The λ_{max} and molecular extinction coefficients (ε) are listed in Table 1. The absorption spectra of two compounds look similar and consist of three absorption bands located at about 320-, 290-, and 250 nm. The higher energy bands undergo practically no shift by solvent changes for both compounds. However, the first band at 320 nm is significantly red-shifted with an increase of polarity and hydrogen bonding ability of the solvent, exhibiting a high molecular extinction coefficient. This indicates that the first absorption maximum band of both compounds originate from a strong $S_o \rightarrow {}^1(\pi, \pi^*)$ transition.

Figure 2 shows the variation of the first $\bar{\nu}_{max}$ of piroxicam plotted as a function of the solvent dielectric constant (D) and refractive index (n) of the solvent, $[(D-1)/(D+2)]-[(n^2-1)/$

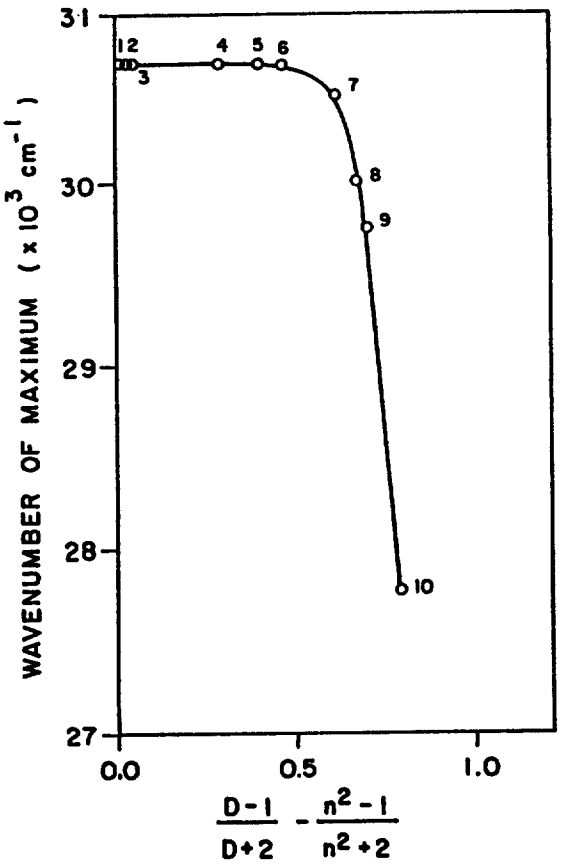


Figure 2. Solvent shifts of 1st absorption band of piroxicam for the solvents: (1) hexane; (2) CCl_4; (3) dioxane; (4) chloroform; (5) ethylacetate; (6) dichlomethane; (7) buthanol; (8) ethanol; (9) methanol and (10) H_2O.

$(n^2+2)]$ as expressed for the transition accompanied by a change in dipole moment[11]. This plot shows that $\bar{\nu}_{max}$ is almost constant with respect to polarity when aprotic solvents are used, but it strongly depends on the hydrogen bonding ability of protic solvents. Especially the red shift is markedly large in water. These indicate that the hydrogen bonding probably plays an important role in the spectral shift.

In nonpolar solvent, the $S_o \rightarrow {}^1(n, \pi^*)$ absorptions of the carbonyls of heterocyclic or aromatic compounds commonly observed in the range 340-370 nm are presumably blue shifted because of intramolecular hydrogen bonding and partially hidden by the stronger $S_o \rightarrow {}^1(\pi, \pi^*)$ absorption as in the case of o-hydroxy benzaldehyde[12]. Supporting this, the first absorption bands exhibit a long and weak tail around 370-380 nm in nonpolar solvents, which disappears in the strong polar hydrogen bonding solvent like water (see Figure 1). This tail is likely to be the $S_o \rightarrow {}^1(n, \pi^*)$ transition which is responsible for the lowest electronic state. The intramolecular hydrogen bonding can take place between the -OH and the carbonyl group of the benzothiazine ring, forming the stable six-member ring in the molecule. In fact, NMR spectra of piroxicam and HMBDC in $CDCl_3$ show a highly deshielding effect for the OH proton and exhibit singlet peak at 13 ppm and 12 ppm, respectively (see experimental), supporting the possibility of the intramolecular hydrogen bonding.

In the strong polar hydrogen bonding solvent, the low-

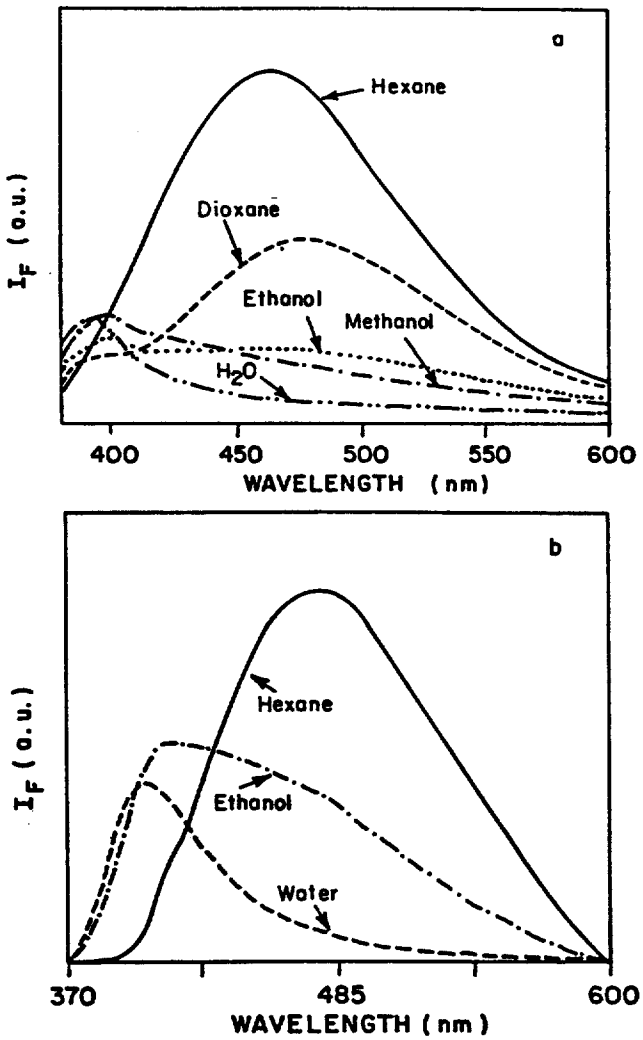


Figure 3. Room temperature fluorescence emission spectra of piroxicam (a) and HMBDC (b) in various solvents. Excitation wavelength was 320 nm or 330 nm.

lying ${}^1(n, \pi^*)$ state would be even further blue shifted by the intermolecular hydrogen bonding while ${}^1(\pi, \pi^*)$ state is red shifted by the high polarity of solvent. Thus the ${}^1(\pi, \pi^*)$ state may become the lowest excited state (S_1) in water. These electronic properties have been observed in many other aromatic carbonyl compounds and N-heterocyclics[13]. The inversion of energy levels would give the ${}^1(\pi, \pi^*)$ state as the emitting state, resulting in increased fluorescence when going from hexane to water. However, this was not observed in our experiment (vide infra), and therefore it suggests that an intramolecular charge-transfer character may be involved in the ${}^1(\pi, \pi^*)$ state. This is consistent with the high molecular extinction coefficient.

Fluorescence properties. Figure 3 shows the fluorescence emission spectra of piroxicam and HMBDC in different solvents. The solvent dependence of fluorescence spectrum of piroxicam (Figure 3a) is generally similar to that of HMBDC (Figure 3b) as in case of the absorption spectra, indicating that the skeleton of both molecules, o-hydroxy benzothiazine carboxylate is important in the solvent dependent fluorescence properties of piroxicam. The fluorescence spectra of both compounds in water show only one narrow emission band of about 400 nm, and their fluorescence quan-

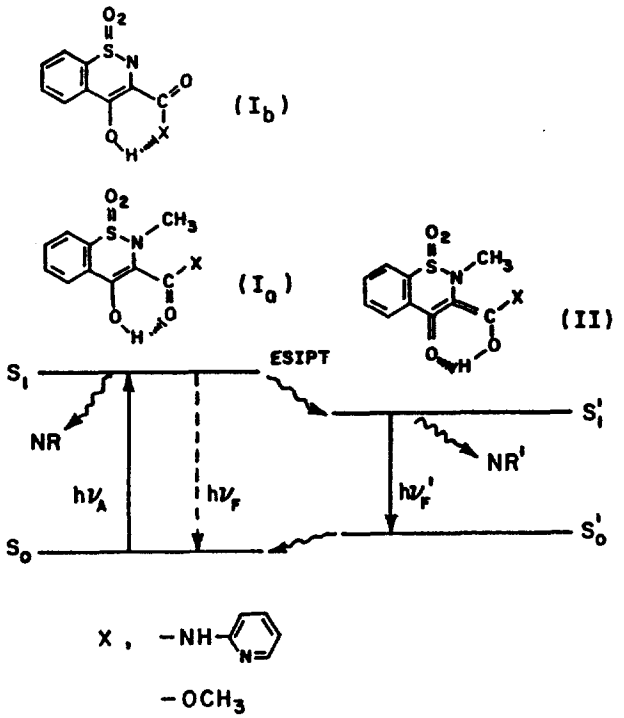


Figure 4. A schematic energy diagram for the dynamic processes of the major species of piroxicam and HMBDC in nonpolar solvents. The straight and wavy lines represent radiative and nonradiative processes, respectively. The dotted line represents the possible fluorescence emission from the primarily excited molecules.

tum yields are very low ($(4.0 \pm 0.2) \times 10^{-3}$) and are even lower than in nonpolar solvent (e.g., $(3.5 \pm 0.1) \times 10^{-2}$ in hexane). The low fluorescence quantum yields are in good agreement with the strong $^1(n, \pi^*)$ or charge-transfer character of the $S_o \longrightarrow S_1$ absorption band. The highly quenched fluorescence in water might be due to the presence of the strong interaction between water and the excited molecules. As in many heterocyclics and aromatic carbonyl compounds[12,13], the strong intermolecular interaction may induce the further closeness of the $^1(n, \pi^*)$ and $^1(\pi, \pi^*)$ state (CT state) or the state switching so that nonradiative transitions are very effective.

As the polarity and hydrogen bonding ability of solvent decrease, the fluorescence spectra become rather broad and are led to progressive appearance of a new red-shifted emission maximum at about 470 nm. The new emission shows the extraordinarily large Stokes' shift (~ 9000 cm^{-1}). Such a solvent dependence is quite a contrast to the normal solvent effect (relaxation) which is usually caused by reorientation of the solvent molecule around the excited dipole[14].

The abnormal solvent dependence of fluorescence spectra may be explained, when considering the formation of a valence isomerized product through the relaxation process such as an excited state intramolecular proton transfer (ESIPT) (see Figure 4). Especially in nonpolar solvents the intramolecular hydrogen bondings between -OH and the ortho carbonyl group in the side chain of benzothiazine ring could greatly favor the ESIPT. In fact, many studies on ESIPT have been done already in ortho-hydroxy aromatic acid or aldehyde derivatives which contain an intramolecular hydrogen bonding between the two neighboring functional group[15-20]. These studies have shown that the excited state

Table 2. Emission Wavelength-Dependence of Fluorescence Lifetimes of HMBDC and Piroxicam in Ethanol at Room Temperature

	Wavelength-dependent lifetimes, ns	
Molecule	410 nm	500 nm
HMBDC	1.3 ± 0.1 (0.6)*	0.4 ± 0.1 (0.8)
	9.4 ± 0.2 (0.4)	9.6 ± 0.4 (0.2)
Piroxicam	0.2 ± 0.1 (0.9)	≤ 0.15
	9.6 ± 0.4 (0.1)	

*The fraction of the component with the lifetime τ_i.

rearrangement of the primary molecule into the tautomeric form occurs through the ESIPT and leads to an abnormally large Stokes' shift of emission maximum in nonpolar solvent. In analogy with the ortho hydroxy aromatic acid or aldehyde derivatives, the primarily excited closed conformer (I_a), of piroxicam or HMBDC (Figure 4) would be relaxed to its enol tautomer (II) which fluoresces at the lowered energy state (S_1^1). The closed conformer (I_a) seems to be main absorbing species for the largely Stokes-shifted emission, since the fluorescence excitation spectra were observed to be in good agreement with the absorption spectra (data not shown).

However, if the hydrogen bonding ability of the solvent is very strong, the intermolecular hydrogen bonding overcomes the intramolecular hydrogen bonding so that open conformer would be the most available species and the ESIPT would not be possible. This may be the reason why the fluorescence spectra in water exhibits only one moderately Stokes-shifted band at about 400 nm. In ethanol, dual emission bands were clearly observed at 400 and 470 nm (Figure 3), indicating that both the closed and open conformers may exist in the moderate hydrogen-bonding solvent. Here it is noteworthy that the relative intensity ratio of the dual emission bands (I_{400}/I_{470}) of piroxicam in ethanol was found to be increased from 1.3 to 1.8 when the solvent was changed with deuterated ethanol-D. This indicates again that the intermolecular hydrogen bonding plays an important role in preventing the formation of the proton-transferred form as suggested for the deuteration effect on ESIPT in 3-hydroxy flavon[21].

In order to further confirm the above arguments, we have measured fluorescence lifetimes of piroxicam and HMBDC for both the moderately Stokes-shifted emission band (410 nm) and the largely Stokes-shifted band (500 nm) by isolating these bands with interference filters (see Experimental) and using the time-correlated single photon counting (SPC) technique. The fluorescence decay curves of both compounds in hexane followed the pulse profile at both emission wavelengths, indicating that the fluorescence lifetimes are shorter than 0.15 ns which is the limit of the apparatus used[10]. However, the fluorescence decay was clearly measurable in ethanol except for the 500 nm emission of piroxicam, indicating that the fluorescence lifetimes are much shorter in nonpolar solvent than in polar protic solvent. These results are consistent with a common property of other intramolecularly hydrogen-bonded molecules that the fluorescence lifetime in nonpolar solvent is short due to a rapid proton transfer followed by fast internal conversion, migrated low-frequency molecular torsional modes[11,22]. The

decay curves of HMBDC in ethanol obtained by observing the emission at 410 nm and 500 nm were well fit to the double exponential decay, exhibiting two lifetime components (Table 2). The fraction of the lifetime component depends on the wavelength at which the emission is detected. In addition, the short lifetime for the 500 nm emission (0.4 ± 0.1 ns) is much smaller than the corresponding value for the 410 nm emission (1.3 ± 0.1 ns).

These results are consistent with the concept that the fluorescent species responsible for the two emission are different from each other as discussed above. The same explanation can be applied to the fact that the fluorescence lifetimes of piroxicam in ethanol could not be measured by observing 500 nm emission, as the lifetime at 410 nm is even smaller (0.2 ns) than that for HMBDC.

In contrast to the short lifetime components, the value of the long component of HMBDC remains similar even when the monitored emission wavelengths are different. At present we cannot make unambiguous assignment of this long component. However one possible explanation can be suggested in terms of two different structures of the closed conformer of HMBDC as proposed for methyl salicylate[23]. One closed conformer (Ia) is expected to be easily converted to the proton-transferred form upon excitation, whereas the other conformer (I_b) would have difficulty for ESIPT (see Figure 4). Thus the rotamer I_b may be partially responsible to the moderately Stokes-shifted emission with long lifetimes. On the other hand, in case of piroxicam, the formation of the I_b-type rotamer may be less favorable because NH is weaker proton acceptor than $-OCH_3$ in HMBDC[23]. This may be the reason why the fraction of the long-lifetime component for 410 nm emission is much smaller than the corresponding fraction in HMBDC. This is also consistent with the observation that the fluorescence spectrum of piroxicam in hexane does not show any shoulder near 400 nm while the shoulder is clearly exhibited for HMBDC (see Figure 3). Nevertheless, further investigations are needed to clarify these points. For this purpose, synthesis of various substituted benzothiazine carboxylate derivatives are underway. Finally, the case of ESIPT in the present N-heterocyclic carboxylate derivatives seems to be analogous to phenomenon in o-hydroxy aromatic carboxylates. However, the detailed dynamic processes in the excited state are open to further investigations, since the dynamic processes associated with the ESIPT are considered to depend on the structural nature of the group attached to the carbonyl group[20,24].

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