Chromatographic determination of narceine imide

B. PROKSA

Slovakofarma, 920 27 Hlohovec

Received 23 March 1981

Geometric isomers of narceine imide were separated and determined by liquid chromatography on columns packed with sorbent with a reversed phase. The stability of *Z*-narceine imide in solution and in solid phase is discussed. Determination of *Z*- and *E*-narceine imides is compared with the results obtained by ^{1}H-n.m.r. spectrometry.

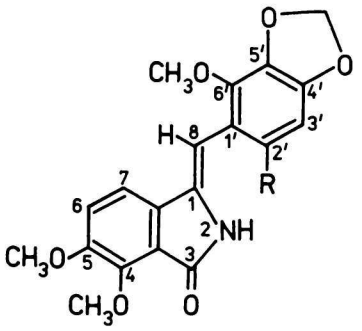
Жидкостной хроматографией на колоннах сорбента с обращенной фазой были разделены и определены геометрические изомеры нарцеинимида. Обсуждается устойчивость *Z*-нарцеинимида в растворе и твердой фазе. Определение *Z*- и *E*-нарцеинимидов при помощи жидкостной хроматографии сравнивается с их определением методом ЯМР ^{1}H.

Narceine imide, isolated from the mixture of minor opium alkaloids [1], was the starting material for preparation of 7,8-dihydro-5*H*-isoindolo[1,2-*b*][3]-benzazepin-5-one [2] derivatives having a specific cytotoxic effect on the lympholeukemia P-388 cells [3]. Due to an asymmetrically substituted double bond C-1—C-8, narceine imide (*I*) and its derivatives (*II*, *III*) exist in two geometrically isomeric forms [1, 4], from which only the *Z*-narceine imide is a suitable material for the synthesis of 7,8-dihydro-5*H*-isoindolo[1,2-*b*][3]benzazepin-5-one.

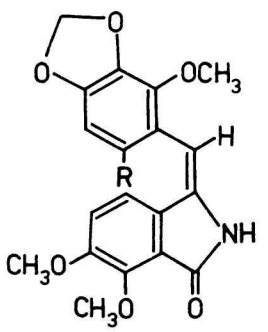
Narceine imide was analytically determined by u.v. and i.r. spectroscopies [5]; nevertheless these methods do not discern between the individual geometric forms.

Geometric isomers can generally be determined by liquid chromatography. Various derivatives of ethylene were separated by adsorption chromatography on silver nitrate impregnated silica gel [6—9], for separation of geometric isomers of cinnamic acid partition chromatography on silica gel applying a ternary two-phase mixture as a mobile phase [9], or chromatography on sorbents with an octadecylsilane-type stationary phase [10] were used.

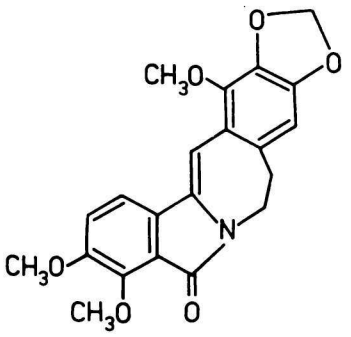
This paper concerns the separation of *Z* and *E* isomers of narceine imide and its derivatives by liquid chromatography using sorbents with a chemically bonded C-18 phase on silica gel. The optimum mobile phase was found to be mixture methanol—water (0.01 mol l^{-1} CH_3COONa) (73:27), flow rate $F = 1$ ml min^{-1}, temperature 40°C. Chromatographic characteristics are listed in Table 1.



I-Z R = $CH_2CH_2N(CH_3)_2$
II-Z R = $CH{=}CH_2$
III-Z R = CH_2CH_3



I-E R = $CH_2CH_2N(CH_3)_2$
II-E R = $CH{=}CH_2$
III-E R = CH_2CH_3



IV

Table 1

Chromatographic characterization of narceine imide derivatives

Parameter	*I-Z*	*I-E*	*II-Z*	*II-E*	*III-Z*	*III-E*
t_R/min	7.40	5.72	4.34	3.26	4.28	3.26
k'	2.64	1.60	2.10	1.33	2.06	1.33
N	876	592	1708	1312	1661	1063
α	1.65		1.57		1.56	
R_s	1.73		2.49		2.49	

t_R — retention time, k' — capacity ratio, N — number of theoretical plates, α — selectivity, R_s — resolution.

The *Z* to *E* ratio of all these compounds in solution and in the light undergoes a time alteration; due to isomerization, the content of the *Z* isomer decreases and consequently, the amount of *E* isomer increases. A like isomerization, but resulting from an energetically substantially wealthier u.v. radiation was observed *e.g.* with derivatives of cinnamic acid [11], or 3-benzylidenephthalimidine [12]. In a diluted solution (0.4 mmol l^{-1}) and within 0—3 h in the light, the decrease of the *Z* form of narceine derivatives can be expressed by an equation of zero order $c_t = c_0 - kt$. The greatest isomerization rate was learned with compound *III-Z* ($k = (1.08 \pm 0.12) \times 10^{-3}$ mmol l^{-1}, the reliability interval of the arithmetic mean value calculated for $\alpha = 0.05$, $n = 5$), a lower rate with *II-Z* ($k = (1.60 \pm 0.13) \times 10^{-3}$ mmol l^{-1}), and the lowest one with narceine imide (*I-Z*) ($k = (7.15 \pm 1.01) \times 10^{-4}$ mmol l^{-1}). Were the solutions of the compounds under study prepared and stored in the dark, only the *Z* form appeared in the chromatogram. The change of *Z* and *E* forms of *II* and *III* was quantitatively evaluated by an internal standard method with propyl 4-hydroxybenzoate and 7,8-dihydro-5*H*-isoindolo[1,2-*b*][3]benzazepin-5-one (*IV*), respectively.

The isomerization of narceine imide can also be well monitored by ^{1}H-n.m.r. spectrometry. Signals of protons (p.p.m., δ scale) of *Z*- and *E*-narceine imides bound to C-6 and C-7 were seen as an AB quartet at 7.67 (d) and 7.08 (d, $J_{6,7} = 8$Hz), and at 6.98 (d) and 6.55 (d), respectively. Integration of signal intensities of the particular peaks followed by a simple calculation offered the *Z* to *E* isomers ratio. The accordance of results of determination of *I*—*E* obtained by liquid chromatography and ^{1}H-n.m.r. spectrometry (26.8 ± 1.3% and 27.6 ± 2.7%, respectively, the reliability interval of the arithmetic mean value calculated for $\alpha = 0.05$ and $n = 5$) was verified by the Student *t* test; as found, the difference in *I*—*E* determination by both methods is statistically insignificant.

Narceine imide has been found in the mixture of opium alkaloids either in the codeine (*VI*), or narcotine (*V*) fractions. These substances were chromatographically separated on the same columns as in preceding measurements, the optimum mobile phase being methanol—dioxan—water (80:5:15). Chromatographic characteristics of the separated compounds are listed in Table 2. The detector response of narceine imide within the 0.01—1.2 mmol l^{-1} concentration was found to be linear. Narceine imide, stored in form of a base for 1 year showed, in addition to the *Z* and *E* forms, another peak. This substance was present in 5.25% and after being isolated, it was identified as 3,4,12-trimethoxy-10,11-methylenedioxy-7,8-dihydro-5*H*-isoindolo[1,2-*b*][3]benzazepin-5-one (*IV*, Ref. [13]). It is our belief that *IV* originated by cyclization of *Z*-narceine imide in solid phase under a release of dimethylamine, which was recognized by a characteristic smell after opening the box with the stored material.

Table 2

Chromatographic characterization of opium alkaloids

Parameter	Compound									
	V		*IV*		*VI*		*I-E*		*I-Z*	
t_R/min	2.15		2.83		3.32		5.03		5.87	
k'	1.40		1.85		2.17		3.28		3.83	
N	929		1454		430		1178		695	
α		1.32		1.17		1.51		1.16		
R_s		2.30		1.31		2.58		1.18		

Experimental

Liquid chromatograph SP-8000 coupled with an u.v. detector SP-770 ($\lambda = 268$ nm) and an integrator SP-4100 (Spectra Physics) was used for chromatographic measurements. Chromatographic column (250 × 4 mm) was packed with Separon Si C-18 (reversed phase, 7—13 μm grain size, Laboratorní přístroje). The dead volume of the column was determined with potassium *p*-toluenesulfonate. The ^{1}H-n.m.r. spectrum was recorded with a Tesla BS 467 (60 MHz) spectrometer in $CDCl_3$ solution containing hexamethyldisiloxane as an internal reference. 1-(6′-Ethenyl-2′-methoxy-3′,4′-methylenedioxy)benzylidene-4,5-dimethoxyisoindolin-3-one (*II*) was obtained by a Hofmann degradation of narceine imide methiodide [13], 1-(6′-ethyl-2′-methoxy-3′,4′-methylenedioxy)benzylidene-4,5-dimethoxyisoindolin-3-one (*III*) was synthesized from *II* by a reduction with hydrazine [4].

Determination of Z- and E-narceine imide

To the methanolic solution of narceine imide (17.5 mg; 4.12×10^{-2} mmol l^{-1}) prepared in the dark the internal standard (propyl 4-hydroxybenzoate, 10 ml, 5×10^{-3} mol l^{-1}) was added and the volume was filled with methanol up to 100 ml; 10 μl of this solution was injected into the chromatograph (mobile phase methanol—water (0.01 mol l^{-1} CH_3COONa) 73:27, flow rate 1 ml min^{-1}, temperature 40°C). The prepared solution, of which the content of the *Z* isomer was prior to this measurement determined, was exposed to the daytime light; samples withdrawn in preset time intervals were analyzed. The content of *Z*- and *E*-narceine imides was calculated employing the already determined response factors; the results are given in Table 3. Linear regression was used for determination of coefficients in the expression $c_t = c_0 - kt$. For the decrease of *Z*-narceine imide from the given initial concentration it holds $c_t = 0.405—7.15 \times 10^{-4} t$ in the 0—210 min time interval. (The reliability intervals of coefficients of the above-mentioned relation: 0.405 ± 0.012; $(7.15 \pm 1.01) \times 10^{-4}$.)

Table 3

Time dependence of a daylight isomerization of *Z*- and *E*- narceine imide

n	*t*/min	C_Z/mmol l^{-1}	C_E/mmol l^{-1}
1	0	0.412 ± 0.020	
2	19	0.386 ± 0.016	0.0220 ± 0.0010
3	44	0.384 ± 0.021	0.0240 ± 0.0011
4	61	0.361 ± 0.014	0.0470 ± 0.0010
5	81	0.350 ± 0.013	0.0582 ± 0.0019
6	99	0.318 ± 0.016	0.0920 ± 0.0030
7	159	0.295 ± 0.012	0.1133 ± 0.0030
8	180	0.272 ± 0.012	0.1359 ± 0.0030
9	210	0.265 ± 0.013	0.1468 ± 0.0048

C_Z — concentration of *Z*-narceine imide; C_E — concentration of *E*-narceine imide.
Reliability interval of arithmetic mean value calculated from 5 parallel determinations for $\alpha = 0.05$.

Analysis of narceine imide stored in form of a base

To the solution of narceine imide (base, 10 mg), stored for 1 year, a solution of an internal standard (narcotine, 10 ml, 4.18 mmol l^{-1}) was added and the volume was filled up to 100 ml with methanol. 10 µl of this solution was injected into the chromatograph (the mobile phase methanol—dioxan—water 80 : 5 : 15, flow rate 2 ml min^{-1}, temperature 40°C). The response factors of the compounds were determined from the calibration graph of the mixture: narcotine 2.09 mmol l^{-1}, *IV* 0.205 mmol l^{-1}, *E*-narceine imide 0.07 mmol l^{-1}, *Z*-narceine imide 0.946 mmol l^{-1}.

References

1. Hodková, J., Veselý, Z., Koblicová, Z., Holubek, J., and Trojánek, J., *Lloydia 35*, 61 (1972).
2. Proksa, B., Votický, Z., and Štefek, M., *Chem. Zvesti 34*, 248 (1980).
3. Fuska, J., Fusková, A., and Proksa, B., *Neoplasma 27*, 703 (1980).
4. Proksa, B. and Votický, Z., *Collect. Czech. Chem. Commun. 45*, 2125 (1980).
5. Proksa, B., Proksová, M., and Molnár, L., *Pharm. Ind. 40*, 1072 (1978).
6. Heath, R. R., Tumlinson, J. H., Doolittle, R. E., and Proveaux, A. T., *J. Chromatogr. Sci. 13*, 380 (1975).
7. Aigner, R., Spitzy, H., and Frei, R. W., *Anal. Chem. 48*, 2 (1976).
8. Heath, R. R., Tumlinson, J. H., and Doolittle, R. E., *J. Chromatogr. Sci. 15*, 10 (1977).
9. Hövermann, W., Rapp, A., and Ziegler, A., *Chromatographia 6*, 317 (1973).
10. Caccamese, S. and Azzolina, R., *Chromatographia 12*, 545 (1979).
11. Hartley, R. D. and Jones, E. C., *J. Chromatogr. 107*, 213 (1975).

12. Marsili, A. and Scartoni, V., *Gazz. Chim. Ital. 102*, 806 (1972).
13. Trojánek, J., Koblicová, Z., Veselý, Z., Suchan, V., and Holubek, J., *Collect. Czech. Chem. Commun. 40*, 681 (1975).

Translated by Z. Votický