THE PREPARATION AND CHARACTERIZATION OF SOME NOVEL FLUORESCENCE LABELS DERIVED FROM 7-SUBSTITUTED 2*H*-1-BENZOPYRANE-2-ONES

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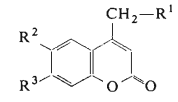
Novel fluorescence compounds derived from 7-substituted 2*H*-1-benzopyrane-2-ones (coumarins) suitable for fluorescence labelling were prepared and characterized.

The interest taken in 7-substituted 2*H*-1-benzopyrane-2-ones (coumarins) and in their spectral properties is due to their physical application (dye lasers[1]) and also to the utilization as optical brighteners. Their utilization in the fluorescence labelling was suggested by Baker and coworkers[2,3]; Murtha[4] chose them as a basis for the preparation of fluorescence labels. The coumarin labels may also be employed in double labelling[5] with other fluorochromes[6]. It has been our aim to prepare and characterize novel fluorescence labels with suitable optical properties, which might also be employed as brighteners for special purposes.

Table I gives a survey of basic data on synthesized compounds. Compound *I* seemed to be the most promising initial fluorochrome, but attempts to prepare it in the reported yield[7] were unsuccessful, in agreement with the literature[8,9]. As its synthesis is rather laborious, the preparation of *XV* became the objective of further work. Baker and coworkers[3] report that they were unsuccessful in trying to prepare the respective hydrazide *XIII* from methyl ester of acid *XI*; the hydrazide was originally suggested by them as an analytical reagent for the labelling of carbonyl compounds with respect to the assumed fluorescence properties of hydrazones derived from it. We attempted the preparation of an analogous amino derivative *XV*. The radial bromination[10] on the methyl group of *II* did not yield the desired bromo derivative which could be transformed into amine. The further procedure was therefore based on the fact that the preparation of the 7-methoxy derivative of hydrazide (*XIII*) had been successful[3]. We therefore prepared the acetyl derivative *XII* of methyl ester which under optimalized conditions gave the required hydrazide *XIII* upon hydrazinolysis. Since the attempt to prepare the N-methacryloylated derivative of hydrazide was unsuccessful, we tried to prepare azide *XIV*, which would be a suitable label for electrophilic groups in the polymer. It was indeed prepared, but proved to be

TABLE I
Basic Data on Synthesized Compounds

Compound (yield, %)	M.p., °C (solvent)	Formula (mol.wt.)	Calculated/Found % C	Calculated/Found % H	IR Spectrum cm^{-1}
I[a] (1)	226[b] (ethanol)	$C_{10}H_9NO_2$[c] (175·2)	68·56 68·48	5·24 5·21	1 544, 1 610, 1 680, 1 690, 3 248, 3 358, 3 442
II[d] (90)	185[e] (acetone)	$C_{10}H_8O_3$ (176·2)	68·18 68·16	4·58 4·55	1 459, 1 515, 1 595, 1 675, 3 160
III (83)	150[f] (methanol)	$C_{12}H_{10}O_4$ (218·2)	66·05 66·10	4·62 4·65	1 508, 1 565, 1 625, 1 710, 1 764
IV (88)	116 (acetone)	$C_{14}H_{12}O_4$ (244·2)	68·85 68·78	4·95 4·94	880, 1 504, 1 573, 1 615, 1 626, 1685, 1 724
V[g] (40)	286[h] (acetone)	$C_{11}H_7ClO_5$[i] (254·6)	51·89 51·91	2·77 2·78	635, 1 685, 1 719
VI (95)	240 (methanol)	$C_{12}H_9ClO_5$[j] (268·7)	53·65 53·62	3·38 3·43	638, 1 510, 1 565, 1 611, 1 703, 1 726
VII (92)	171 (acetone)	$C_{14}H_{11}ClO_6$[k] (310·7)	54·12 54·20	3·57 3·66	640, 1 558, 1 610, 1 624, 1 728, 1 766
VIII[l] (85)	210[m] (acetone)	$C_{11}H_8O_5$ (220·2)	60·00 60·04	3·66 3·67	1 517, 1 561, 1 615, 1 697
IX (85)	175 (methanol)	$C_{13}H_{10}O_6$ (262·2)	59·55 59·48	3·84 3·78	1 504, 1 570, 1 610, 1 622, 1 725
X (62)	149 (acetone)	$C_{15}H_{12}O_6$ (288·3)	62·50 62·46	4·20 4·22	887, 1 503, 1 561, 1 614, 1 638, 1 690, 1 728
XI (91)	220[n] (methanol)	$C_{12}H_{10}O_5$ (234·2)	61·54 61·52	4·30 4·34	1 521, 1 602, 1 689, 1 721
XII (90)	155 (methanol)	$C_{14}H_{12}O_6$ (276·2)	60·87 60·79	4·38 4·33	1 505, 1 569, 1 617, 1 722, 1 754
XIII (75)	284[o]	$C_{11}H_{10}N_2O_4$[p] (234·2)	56·41 56·38	4·30 4·30	1 513, 1 550, 1 562, 1 603, 1 619, 1 637, 1 706, 3 320
XV (85)	253[o]	$C_{10}H_9NO_3$[r] (191·2)	62·82 62·58	4·74 4·83	1 587, 1 600, 1 612, 1 739, 3 287, 3 342
XVI (34)	248[o]	$C_{10}H_{10}ClNO_3$[s] (227·6)	52·76 52·65	4·43 4·49	1 498, 1 565, 1 611, 1 716
XVII (30)	218[o]	$C_{16}H_{12}N_4O_{10}$[t] (420·3)	45·72 45·57	2·88 2·92	1 360, 1 520, 1 550, 1 583, 1 610, 1 706
XVII (65)	246 (acetone)	$C_{14}H_{13}NO_4$[u] (259·3)	64·86 64·69	5·05 5·09	894, 1 514, 1 538, 1 590, 1 615, 1 646, 1 724



	R^1	R^2	R^3
I,	H	H	NH_2
II,	H	H	OH
III,	H	H	$OCOCH_3$
IV,	H	H	$OCOC(CH_3)=CH_2$
V,	COOH	Cl	OH
VI,	$COOCH_3$	Cl	OH
VII,	$COOCH_3$	Cl	$OCOCH_3$
VIII,	COOH	H	OH
IX,	COOH	H	$OCOCH_3$
X,	COOH	H	$OCOC(CH_3)=CH_2$
XI,	$COOCH_3$	H	OH
XII,	$COOCH_3$	H	$OCOOCH_3$
XIII,	$CONHNH_2$	H	OH
XIV,	CON_3	H	OH
XV,	NH_2	H	OH
XVI,	NH_3Cl	H	OH
XVII,	$NH_3OC_6H_2(NO_2)_3$	H	OH
XVIII,	$NHCOC(CH_3)=CH_2$	H	OH

unstable and would have to be used as the fluorescence label immediately after its preparation. For this reason, azide *XIV* was transformed by the Curtius reaction into the stable amine *XV*, which had already been successfully used in the preparation of labelled polyaspartamide[5] by means of a polymeranalogous reaction. Owing to the low solubility of *XV*, salts *XVI*, *XVII* were prepared, which confirmed the

[a] Prepared according to[7]. [b] °C ref.: 226[7], 220–224[9], 222–223[8]. [c] % N calc./found: 7·99/7·90. [d] Prepared according to[12]. [e] Ref.[12] 185–186°C. [f] Ref.[12] 150°C. [g] Prepared according to[14]. [h] °C ref.: 210[13], 212[14], 286–287[15]. [i] % Cl calc./found: 13·93/13·98. [j] % Cl calc./found: 13·20/13·28. [k] % Cl calc./found: 11·47/11·34. [l] Prepared according to[3]. [m] °C ref.: 183·5–185[16], 198[17], 200[18], 201[19], 201–202[20,21], 203–204[22], 210[3], 210–212[23]. [n] Ref.[3] 220°C. [o] The compound melts with decomposition, m.p.'s are hardly reproducible. [p] % N calc./found: 11·96/11·89. [r] % N calc./found: 7·33/7·19. [s] calc./found: % N 6·15/6·15; % Cl 15·58/15·45. [t] % N calc./found: 13·33/13·23. [u] % N calc./found: 5·40/5·32.

TABLE II

^{1}H-NMR Data of Synthesized Compounds (R^1, R^3 *cf.* Scheme)

Compound	Chemical shifts (ppm) and interaction constant J (Hz) in positions						
	3	5	6	8	$-CH_2-$	R^1	R^3
I[a]	4·11 q $J_{3CH_3} = 1$	2·60 d $J_{56} = 8$	3·41 q $J_{68} = 2·4$ $J_{65} = 8$	3·60 d $J_{86} = 2·4$		7·68 d $J_{3CH_3} = 1$	3·91
II[a]	3·92 q $J_{3CH_3} = 1·1$	2·43 s $J_{56} = 8·5$	3·20 q $J_{68} = 2·4$ $J_{65} = 8·5$	3·30 d $J_{86} = 2·4$		7·67 d $J_{3CH_3} = 1·1$	−0·51
III[b]	3·66 q $J_{3CH_3} = 1·1$	2·20 d $J_{56} = 8·5$	2·84 q $J_{68} = 2·5$ $J_{65} = 8·5$	2·78 d $J_{86} = 2·5$		7·55 d $J_{3CH_3} = 1·1$	7·67 s
IV[a]	3·62 q $J_{3CH_3} = 1·5$	2·16 d $J_{56} = 8·5$	2·78 q $J_{68} = 2·5$ $J_{65} = 8·5$	2·64 d $J_{86} = 2·5$		7·56 d $J_{3CH_3} = 1·5$	3·69 q (H *trans*) J_{CH_3H} (*trans*) = 1·2 4·08 q (H *cis*) J_{CH_3H} (*cis*) = 1·7 7·99 m (CH_3)
V[a]	3·74 s	2·32 s	Cl	3·12 s	6·14 s		—
VI[b]	3·72 s	2·28 s	Cl	3·08 s	6·07 s	6·30 s	−1·17
VII[b]	3·45 s	2·05 s	Cl	2·52 s	5·95 s	6·32 s	7·62 s
VIII[a]	3·85 s	2·50 d $J_{56} = 8·5$	3·24 q $J_{68} = 2·4$ $J_{65} = 8·5$	3·28 d $J_{86} = 2·4$	6·18 s		

IX[a]	3·50 s	2·24 d $J_{56} = 8$	2·81 q $J_{68} = 2{\cdot}4$ $J_{65} = 8$	2·69 d $J_{86} = 2{\cdot}4$	6·05 s		7·70 s
X[a]	3·55 s	2·27 d $J_{56} = 8{\cdot}5$	2·82 q $J_{68} = 2{\cdot}5$	2·68 d $J_{86} = 2{\cdot}5$	6·08 s		3·71 q (H *trans*) J_{CH_3H} (*trans*) = 1·2 4·12 q (H *cis*) J_{CH_3H} (*cis*) = 1·5 7·95 m (CH_3)
XI[b]	3·78 s	2·45 d $J_{56} = 8$	3·18 q $J_{68} = 2{\cdot}4$ $J_{65} = 8$	3·23 d $J_{86} = 2{\cdot}4$	6·10 s	6·35 s	−0·38
XII[b]	3·53 s	2·23 d $J_{56} = 8$	2·82 q $J_{68} = 2{\cdot}4$ $J_{65} = 8$	2·75 d $J_{86} = 2{\cdot}4$	6·00 s	6·34 s	7·70 s
XIII[a]	3·82 s	2·34 d $J_{56} = 8{\cdot}5$	3·18 q $J_{68} = 2{\cdot}4$ $J_{65} = 8{\cdot}5$	3·25 d $J_{86} = 2{\cdot}4$	6·45 s	0·70 (NH) 5·65 (NH_2)	−0·60
XVI[a]	3·67 s	2·32 d $J_{56} = 8$	3·12 q $J_{68} = 2{\cdot}4$ $J_{65} = 8$	3·18 d $J_{86} = 2{\cdot}4$	5·70 s	1·10	0·95
XVIII[a]	4·07 s	2·28 s $J_{56} = 8{\cdot}5$	3·21 q $J_{68} = 2{\cdot}4$ $J_{65} = 8{\cdot}5$	3·30 d $J_{86} = 2{\cdot}4$	5·55 d $J_{CH_2NH} = 6{\cdot}0$	4·25 q (H *trans*) J_{CH_3H} (*trans*) = 1·0 4·60 q (H *cis*) J_{CH_3H} (*cis*) = 1·9 8·10 m (CH_3) 1·45 t (NH) $J_{CH_2NH} = 6{\cdot}0$	−0·60

The measurement was carried out at [a] 22°C, [b] 80°C.

structure of *XV*. *XV* readily yielded the N-methacryloyl derivative *XVIII*, a suitable fluorescence label for the preparation of fluorescence-labelled polymers by copolymerization.

All attempts to prepare analogous 6-chloro derivatives have failed.

Both 7-methacryloyl derivatives, *VII* and *X*, are not of much value as fluorescence labels because of the weak intensity of fluorescence and hydrolytic instability.

An attempt to obtain amine derivative by reacting *VIII* and *IX* with ethylenediamine and hexamethylenediamine by means of dicyclohexylcarbodiimide failed, because the reaction did not proceed in the desired direction; the decarboxylated 4-methyl derivative *II* was isolated from the reaction mixture. The thermal gravimetric analysis (TGA) method showed that the decarboxylation of *VIII* in the solid state occurs already at 51°. The easy decarboxylation of 4-carboxy methyl derivatives of 2*H*-1-benzopyrane-2-one is likely to be the main cause of discrepancies between the melting points of these compounds reported in the literature. Compounds for which no melting points are given in Table I melt with decomposition. The synthesized compounds are better characterized by employing their spectral data (IR *cf.* Table I, ^{1}H-NMR *cf.* Table II).

Tables III and IV summarize spectral absorption and fluorescence data of compounds under study related to 4-methyl-7-hydroxy-2*H*-1-benzopyrane-2-one (*II*) in the nonionized state.

The fluorescence intensities were compared[11] by using the calculated ratio of quantum yield derivatives at the emission maximum, Φ'_r:

$$\Phi'_r = \frac{\left(\frac{\Delta\Phi}{\Delta\nu}\right)_{\nu_i}}{\left(\frac{\Delta\Phi}{\Delta\nu}\right)_{\nu_s}} = \frac{S_{i\lambda} \cdot H^S_{i\lambda} \cdot S^{Rgh}_{s\lambda} \cdot H^S_{s\lambda Rgh} \cdot \hat{\lambda}^5_{s,Rgh} \cdot \lambda_i \cdot c_s \cdot \hat{\varepsilon}_s}{S_{s\lambda} \cdot H^S_{s\lambda} \cdot S^{Rgh}_{i\lambda} \cdot H^S_{i\lambda Rgh} \cdot \hat{\lambda}^5_{i,Rgh} \cdot \lambda_s \cdot c_i \cdot \hat{\varepsilon}_i},$$

in which c is concentration (mol l^{-1}), ε is the molar extinction coefficient, λ is the wavelength (nm), ν is the wave number (cm^{-1}), S is the magnitude of signal of the apparatus (scale divisions), H^S is the calibration function of the apparatus, Φ is the quantum yield; the indices have the following meaning: s is the standard (*II* in our case), i is the compound being compared, Rgh is the Rayleigh scattering, $\wedge$ is a value at the wavelength of excitation. The Rayleigh light scattering value was taken as the internal standard of the apparatus. If one wanted to obtain the relative quantum yield $\Phi_r = \Phi_i/\Phi_s$, it would be necessary to sum the products $S_\lambda \cdot H^S_\lambda \cdot \lambda$ for the densest possible distribution of λ. Since for the majority of recorded emission spectra it holds that their shapes are very similar and that they lie roughly in the same range, a semiquantitative estimate can be made by using ratio of quantum yield derivatives at the emission maximum, Φ'_r.

For *I* in the ionized state and for *V* and *VI* in the nonionized states Φ'_r could not be calculated, because their emission spectra differ to much from that of the standard. The fluorescence spectra of 7-acyloxy derivatives were not recorded, because these

compounds are hydrolytically very labile, and yield products having their quantum yield higher by several orders of magnitude.

It may be concluded, that the relatively easy preparation, availability of starting compounds and adequate fluorescence properties render promising the utilization of *XIII*, *XIV* and *XV* in the fluorescence labelling of polymers by the polymer-analogous reaction, and the use of *XVIII* in the fluorescence labelling by copolymerization.

TABLE III

Absorption Spectra of Synthesized Compounds in Methanol

Compound	Pure methanol		Saturated NH_3	
	λ (nm)	$\varepsilon . 10^{-4}$ (l mol^{-1} cm^{-1})	λ (nm)	$\varepsilon . 10^{-4}$ (l mol^{-1} cm^{-1})
I	353	1·81	308[a]	0·72[a]
			266	1·07
II	323	1·53	365	2·02
III	314	0·91	—	—
	280	0·99		
IV	313	1·01	—	—
	279	1·10		
V	333	1·41	371	2·03
VI	331	1·42	—	—
VII	323	0·71	—	—
	274	0·94		
VIII	327	1·47	368	1·90
IX	313	0·90	—	—
	280	0·97	—	—
X	313	1·02	—	—
	281	1·12		
XI	327	1·46	—	—
XII	313	0·89	—	—
	279	0·97		
XIII	325	1·44	371	1·91
XV	322	1·45	365	1·92
XVI	326	1·36	—	—
XVIII	323	1·41	368	1·90

[a] The spectra were recorded in anhydrous methanol saturated with HCl.

EXPERIMENTAL

The melting points were determined with an SMP-20 Büchi in an evacuated sealed capillary. The infrared spectra were recorded with a Zeiss UR 20 spectrometer in KBr tablets. The ^{1}H-NMR spectra were recorded with a JEOL-JNM-PS 100 apparatus in deuterated dimethyl sulphoxide $(CD_3)_2SO$ with hexamethyldisiloxane as the internal standard. The interaction constants J were read off from spectra of 1st order. The UV spectra were recorded with a Cary 14 apparatus. The TGA analysis was performed with an apparatus built at the Institute at the heating rate 1°C/min. An apparatus built at the Institute was also used in recording the fluorescence spectra[11], with automatically corrected excitation spectra.

7-Acetoxy-4-methyl-2*H*-1-benzopyrane-2-one (*III*)

77 g (0·437 mol) *II* and 500 ml acetanhydride were refluxed for 2 h and dried over KOH *in vacuo*. 6-Chloro-7-acetoxy-2-oxo-2*H*-1-benzopyrane-4-acetic acid methyl ester (*VII*), 7-acetoxy-2-oxo-2*H*-1-benzopyrane-4-acetic acid (*IX*) and 7-acetoxy-2-oxo-2*H*-1-benzopyrane-4-acetic acid methyl ester (*XII*) were prepared similarly to *III*.

TABLE IV

Fluorescence Spectra of Synthesized Compounds in Methanol

Compound	Pure methanol			Saturated NH_3		
	λ_{exc} nm	λ_{em} nm	Φ'_r	λ_{exc} nm	λ_{em} nm	Φ'_r
I	352	430	2·75	307[a]	475[a]	[b]
II	321	380	1·00	369	447	1·32
V	331	393 492	[b]	374	453	2·68
VI	330	393 492	[b]	—	—	—
VIII	326	385	0·59	371	457	0·89
XI	326	389	1·16	—	—	—
XIII	327	390	0·25	376	454	2·15
XV	325	387	0·48	369	458	1·36
XVI	324	392	0·40	—	—	—
XVIII	325	392	0·18	373	445	1·90

[a] The spectra were recorded in anhydrous methanol saturated with HCl. [b] Φ'_r could not be determined because the emission spectra exhibit a complicated character

7-Methacryloyloxy-4-methyl-2*H*-1-benzopyrane-2-one (*IV*)

8g (0·2 mol) of NaOH and 35·24 g (0·2 mol) of *I* were dissolved in 800 ml of water. On cooling to 0°C, 19·2 ml (0·2 ml) of methacryloyl chloride were added dropwise with stirring, and the stirring was continued at 0°C for another 2 h. The precipitate was washed with water, digested in 5% NaOH, filtered, washed with dilute HCl and eventually with water.

Methyl esters of 6-chloro-7-hydroxy-2-oxo-2*H*-1-benzopyrane-4-acetic acid (*VI*) and of 7-hydroxy-2-oxo-2*H*-1-benzopyrane-4-acetic acid (*XI*) were prepared according to Fischer.

7-Methacryloyloxy-2-oxo-2*H*-1-benzopyrane-4-acetic Acid (*X*)

16 g (0·4 mol) NaOH and 44·04 g (0·200 mol) *VIII* were dissolved in 800 ml water, cooled to 0°C, and 19·2 ml (0·200 mol) of methacryloyl chloride was added dropwise with stirring. The stirring continued at 0°C for 2 h. The product was separated by acidifying the solution with 5% HCl, and the precipitate was dissolved in sodium hydrogen carbonate. *X* was obtained by acidifying with 5% HCl and dried over P_2O_2 *in vacuo*.

7-Hydroxy-2-oxo-2*H*-1-benzopyrane-4-acetic Acid Hydrazide[24] (*XIII*)

15 g (0·054 mol) was dissolved in a solution containing 600 ml methanol and 33 ml of an 80% solution of hydrazine hydrate and left to stand over night. The precipitate was filtered by suction, washed with methanol and light petroleum, and recrystallized from water.

7-Hydroxy-2-oxo-2*H*-1-benzopyrane-4-acetic Acid Azide (*XIV*)
and 4-Aminomethyl-7-hydroxy-2*H*-1-benzopyrane-2-one[25] (*XV*)

28 g *XIII* (0·120 mol) was dissolved while hot in 1 l water containing 60 ml conc. HCl. The solution was cooled, and a solution of 16 g (0·232 mol) $NaNO_2$ in 100 ml water was added. The precipitate of azide *XIV* appeared almost immediately. The mixture was left overnight at −15°C. The precipitate was then dissolved in a solution of 800 ml water and 40 ml conc. HCl and boiled under reflux for one hour. The filtrate was neutralized with sodium hydrogen carbonate and left overnight. The precipitate was digested while hot with *c.* 1 l water and 1 l methanol.

4-Methacryloylaminomethyl-7-hydroxy-2*H*-1-benzopyrane-2-one (*XVIII*)

9·6 g (0·240 mol) NaOH and 22·94 g (0·120 mol) *XV* were dissolved in 480 ml water. The solution was cooled to 0°C, and 11·54 ml (0·120 mol) methacryloyl chloride was added dropwise with stirring; the stirring continued for another 30 min. The filtrate was cooled, acidified with a 10% HCl, the precipitate was filtered and dried over P_2O_5 *in vacuo*.

REFERENCES

1. Řehák V.: Chem. Listy *70*, 1 (1976).
2. Baker W., Collis C. B.: J. Chem. Soc. *1949*, 12.
3. Baker W., Haksar C. N., McOmie J. F. W.: J. Chem. Soc. *1950*, 170.
4. Murtha T. P.: *Thesis*. Iowa State University of Science and Technology, Ames, Iowa 1968.
5. Rypáček F., Drobník J., Krejčoves J., Kálal J.: J. Polym. Sci., in press.
6. Krejčoves J., Drobník J., Kálal J.: Chem. Listy *73*, 363 (1979).
7. Pechmann H., Schwarz O.: Ber. Deut. Chem. Ges. *32*, 3696 (1899).
8. Zimmerman M., Yurewicz E., Patel G.: Anal. Biochem. *70*, 258 (1976).

9. Atkins R. L., Bliss D. E.: J. Org. Chem. *43*, 1975 (1978).
10. Ghiya B. J., Marathey M. G.: J. Indian Chem. Soc. *42*, 229 (1965).
11. Krejčoves J.: *Thesis*. Czechoslovak Academy of Sciences, Prague 1978.
12. Leonhardi G., Glasenapp I.: Hoppe-Seyler's Z. Physiol. Chem. *286*, 145 (1950).
13. Chakravarti D.: J. Indian Chem. Soc. *12*, 622 (1935).
14. Kansara H. P., Shah N. M.: J. Univ. Bombay *17A*, 57 (1948); Chem. Abstr. *43*, 6206 (1949).
15. Woods L. L., Sapp J.: J. Chem. Eng. Data *8*, 235 (1963).
16. Calco J. M., Bartholomew J. C., Stieglitz B. I.: Anal. Biochem. *28*, 164 (1969).
17. Mangini A., Passerini R.: Gazz. Chim. Ital. *87*, 243 (1957).
18. Eswaranarayana N.: Rec. Trav. Chim. Pays-Bas *72*, 1003 (1953).
19. Dey B. B., Row K. K.: Quart. J. Indian Chem. Soc. *1*, 107 (1924); Chem. Abstr. *19*, 823 (1925).
20. Burton B. S.: Ann. N. Y. Acad. Sci. *261*, 167 (1891).
21. Laskowski S. C., Clinton R. O.: J. Amer. Chem. Soc. *72*, 3987 (1950).
22. Kotwani N. G., Sethna S. M., Advani G. D.: Proc. Indian Acad. Sci *15A*, 441 (1942).
23. Bose P. K., Sen P. B., Chakravarti K.: Ann. Biochem. Exptl. Med. *5*, 1 (1945).
24. Krejčoves J., Drobník J., Kálal J.: AO 187 539. 8. 9. 78.
25. Krejčoves J., Drobník J., Kálal J.: AO 187 540. 8. 9. 78.

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