Tetrahedron, 1966, Vol. 22, pp. 1153 to 1158. Pergamon Press Ltd. Printed in Northern Ireland

STUDIES ON PLANTS—X[1,2]

BEHAVIOUR OF RIBALINIUM SALTS TOWARDS BASES

R. A. CORRAL and O. O. ORAZI
Facultad de Química y Farmacia, Universidad Nacional de La Plata, La Plata, Argentina

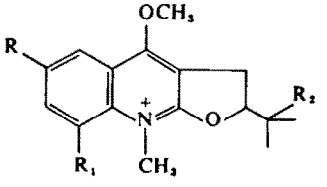
(*Received* 17 *September* 1965)

Abstract—Departing from structurally related quaternary compounds, the phenolic ribalinium ion (Ia) does not suffer opening of the dihydrofuranic ring under mild basic conditions due to conversion to a resonance stabilized anhydronium system (II).

The stereochemical results in the ring-opening of O^6-methylribalinium (Id) in buffer pH 10-6 and of Ia in stronger basic medium, suggest a dual reaction mechanism. The epoxide IV, an intermediate in a proposed route, has been isolated when working under conditions that prevent its hydrolysis.

RIBALINIUM (Ia) chloride[1] is the only phenolic member of a small group of quaternary linear dihydrofuroquinoline alkaloids occurring in rutaceous species. While Ia is essentially the only quaternary compound present in *Balfourodendron riedelianum* from Argentina, the O^4-methylbalfourodinium (Ib) was isolated in high yield from the same species growing in Brazil;[3] the remaining alkaloid of this type, named lunasine or methyllunacrinium (Ic), was found in the *Lunasia* genus.[4]

There are described in the literature several synthetic quaternary compounds, closely related to the above mentioned alkaloids, prepared in connection with structural studies of some tertiary bases of the Rutaceae family.[3,5,6]



	R	R_1	R_2
Ia	OH	H	OH
Ib	H	OCH_3	OH
Ic	H	OCH_3	H
Id	OCH_3	H	OH

A remarkable feature of these quaternary compounds is their sensitivity to bases which produce the opening of the dihydro-furanic or pyranic ring leading to 2-quinolones.

Although a wide range of basic conditions were used by different authors, it is apparent that the ring-opening may be accomplished even in very mild alkaline medium. For this reason, special precautions in the processing of plants extracts have been taken;[3,7] otherwise, quaternary compounds of this type can be partially or

[1] Part IX: R. A. Corral and O. O. Orazi, *Tetrahedron* **21**, 909 (1965).

[2] We acknowledge generous support of this work by the Consejo Nacional de Investigaciones Científicas y Técnicas.

[3] H. Rapport and K. G. Holden, *J. Amer. Chem. Soc.* **81**, 3738 (1959); **82**, 4395 (1960).

[4] J. R. Price, *Austral. J. Chem.* **12**, 458 (1959).

[5] S. Goodwin, J. N. Shoolery and E. C. Horning, *J. Amer. Chem. Soc.* **81**, 3736 (1959).

[6] H. C. Beyerman and R. W. Rooda, *Proc. Koninkl. Nederl. Akad. Wetensch.* **B-63**, 154 (1960).

[7] R. Johnstone, J. R. Price and A. R. Todd, *Austral. J. Chem.* **11**, 562 (1958).

totally degraded to 2-quinolones as artifacts.[8] An example, is the isolation of balfourolone[3] when the extract is brought to pH 10.

In the case of ribalinium cation (Ia), the same results were obtained whether the alkaloidal extract was processed at pH 6·5 or 11;[1] and this striking stability was confirmed when Ia was recovered unaltered after three days at pH 10·6.

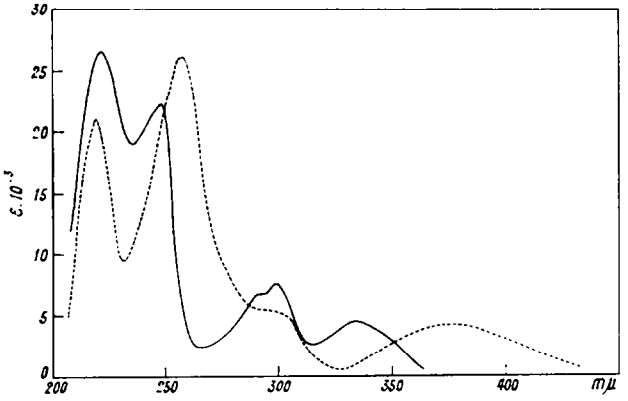
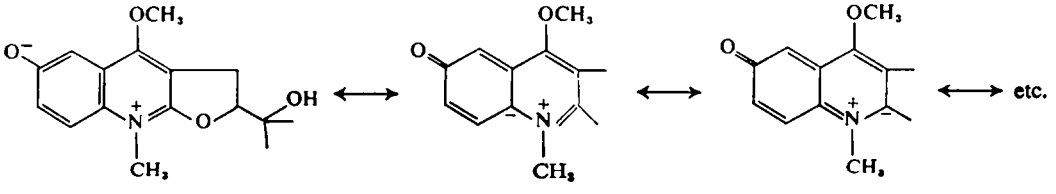


FIG. 1. UV spectra of Ribalinium (Ia) chloride in alcohol 50°:
——— neutral; – – – 0·01N $NaHCO_3$.

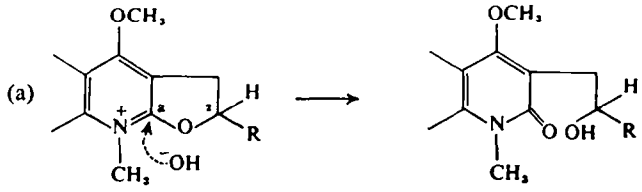
The UV absorption of Ia is substantially modified in shifting from neutral medium to pH 10·6 or even lower as in sodium bicarbonate solution (Fig. 1). The bathochromic displacement is so pronounced that the absorption reaches the visible region; this effect is reversed by neutralization. These results and the pK_a value (8·05 in water, uncorrected) of Ia chloride strongly suggest that in basic medium the phenolic proton of the latter is easily removed leading to a resonance-stabilized anhydronium system. This was substantiated by the isolation of the yellow, beautifully crystalline, xantho-ribalinium (II), the properties of which are typical for the class of compounds.[9]



IIa IIb IIc

The mechanism proposed[4] for the ring-opening of Ic, based on that given for the tertiary lunacrine,[10] involves attack at position-a by an hydroxyl ion while the group attached at this centre is split off. This behaviour is general for quinolinium salts bearing electron-withdrawing substituents at position-2, in reactions with nucleophilic reagents.[11]

[8] S. Goodwin, A. F. Smith, A. A. Velásquez and E. C. Horning, *J. Amer. Chem. Soc.* **81**, 6206 (1959).
[9] For a recent review on anhydronium compounds, see J. P. Saxena, *J. Sci. Ind. Res. India* **22**, 81 (1963).
[10] S. Goodwin and E. C. Horning, *J. Amer. Chem. Soc.* **81**, 1908 (1959).
[11] G. T. Pilyugin and B. M. Gutsulyak, *Russ. Chem. Rev.* **32**, 167 (1963).



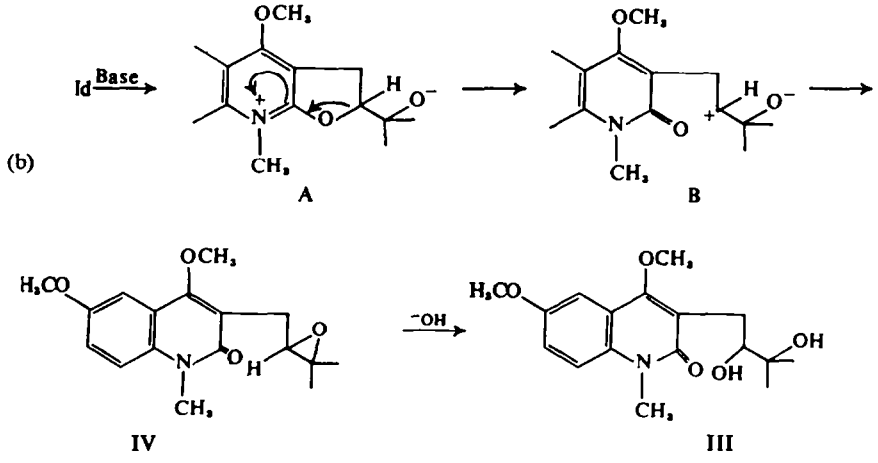
This mechanism explains the reluctance of II and its progenitor Ia to cleave under the above mentioned conditions: attack by hydroxyl ion is rendered difficult because the electrophilicity at carbon-a has been reduced by the contribution of resonance-structure IIc.

This explanation requires that compounds resulting from blockage of the phenolic function should be sensitive to bases. In fact, the hydrolytic opening of O^6-methyl-ribalinium (Id) iodide was complete after 24 hr at room temperature in buffer pH 10·6 leading to the O^6-methylribalone (III). The latter possesses the expected 2-quinolone structure as demonstrated by analytical and spectroscopic data. The IR shows a minimum between 1504 and 1577 cm^{-1} and the UV, with a maximum at 276 mμ, remains unaltered on acidification; its NMR spectrum (in $CDCl_3$ at 100 Mc)[12] exhibits a multiplet centred at δ 7·3 ppm (3 aromatic H) covering a region of only 30 c/s.[13]

The nature of the side chain of III was elucidated as follows: the NMR spectrum displays all the resonance frequencies corresponding to the side chain of hydroxy-lunacridine[5] and the oxidation with sodium periodate affords acetone and the new 1-methyl-3-(β-oxoethyl)-4,6-dimethoxy-2-quinolone isolated as 2,4-dinitrophenyl-hydrazones.

In agreement with mechanisms (a) the ring-opening of Ic takes place with complete retention of the C-2 configuration.[4] From O^6-methylribalinium (Id) iodide we have isolated (−) and (±)-O^6-methylribalone (III), the ratio of the formed laevo and dextro enantiomers being 1·9:1.

This stereochemical result clearly indicates that in our case the ring opening process is more complex. In addition to mechanism (a), another one including the



[12] Run by Varian Assoc., Palo Alto, U.S.A.

[13] Original papers quoted in Ref. 1.

participation of the side-chain hydroxyl must also operate; a possibility is outlined in (b) involving the formation of the intermediate epoxide IV through a SN_1 type process.

The electronic displacements indicated on A leading to B are favoured by the adjacent, strong electron-releasing, charged oxygen atom. The partially or completely racemic epoxide (IV) would be readily converted into the O^6-methylribalone (III) through a SN_2 attack[14] by an hydroxyl anion.[15]

Working under conditions designed to avoid the hydrolysis of IV to III, the isolation of the assumed intermediate epoxide (IV; racemic) was achieved. For this preparation, a solution of O^6-methylribalinium (Id) iodide in N,N-dimethylformamide was treated under anhydrous conditions with sodium hydride which initiates the process forming the alkoxide (A).

The structure of IV is proved by analytical values, absence of hydroxyl absorption in the IR, NMR signal of one epoxide-proton centred at δ 2·97 ppm and its 2-quinolone nature is indicated by NMR, IR and UV data[16] (see above). When the epoxide (IV) is subjected to the hydrolytic conditions used for Id, rapid conversion into III takes place.

It should now be pointed out that also in terms of mechanism (b), the contribution of IIc may easily account for the ring-stability of ribalinium (Ia) in buffer pH 10·6. In stronger alkaline medium, quantitative degradation of Ia takes place; the structure of the resulting phenolic product, ribalone (V), is established by analytical data, IR and UV spectra and methylation to O^6-methylribalone (III). The ratio of the formed laevo and dextro enantiomers of V is 4·4:1; the stereochemistry of the ring-opening suggests that also in this case a dual reaction mechanism is operating.

EXPERIMENTAL

The m.ps, taken in sealed capillaries, were not corrected. The UV spectra were measured in neutral, acidic (0·3N HCl) or basic (0·01N $NaHCO_3$) medium using alcohol 50° as solvent; IR spectra were taken in Nujol mulls, unless otherwise stated. NMR values are given as δ (ppm) downfield from internal $SiMe_4$($\delta = 0$). Optical rotations were determined on methanolic solutions in one-decimeter tube employing a Rudolph polarimeter model 80. The microanalyses were carried out by Dr. A. Bernhardt (Mulheim, Germany).

Interconversion of ribalinium (Ia) *and xantho-ribalinium* (II)

(a) Solution of Ia chloride[1] (0·001 mole) in 3 ml water developed a deep yellow colour on addition of $NaHCO_3$ (0·001 mole); the solvent was removed *in vacuo* adding benzene to eliminate the last traces of water. Several extractions with abs EtOH (or CH_2Cl_2) and subsequent evaporation of the combined extracts led to a quantitative yield of crude *xantho-ribalinium* (II). Crystallization from water–acetone provided fine yellow needles, m.p. 99·5–101°, fairly soluble in water and practically insoluble in benzene, ethyl ether and ethyl acetate. This substance was dried to const. wt. (65°/high vac.) immediately prior to analysis; under these conditions a change of colour to orange is observed (partial loss of crystallization water) which reverts to yellow by moisture uptake. (Found: C, 62·65; H, 6·65; N, 4·69; O, 25·95. $C_{16}H_{19}NO_4.H_2O$ requires: C, 62·52; H, 6·89; N, 4·56; O, 26·03%.)

[14] R. E. Parker and N. S. Isaacs, *Chem. Rev.* **59**, 737 (1959).

[15] Mechanism (b) may also account for the unexplained racemization found[6] in the basic cleavage of the hydroxylated dihydropyranic ring of the quaternary salt derived from lunasia (II).

[16] The significant difference in the UV spectra of III and IV is probably due to the occurrence in the former of intramolecular hydrogen-bonds between the carbonyl and hydroxyl groups. This is also reflected in the position of the first maximum in the IR carbonyl region (Nujol); that of III (1632 cm^{-1}) appears at lower frequency than in IV (1645 cm^{-1}) [Cf. J. R. Price and J. B. Willis, *Austral. J. Chem.* **12**, 589 (1959)].

The same compound (II) crystallized out treating a conc. aqueous solution of Ia chloride with KBH_4 and was also obtained on passing a methanolic solution of Ia through a column of a basic resin.

On allowing Ia chloride or perchlorate dissolved in buffer pH 10·6 (borax–sodium carbonate) to stand for 3 days, tertiary bases extractable by organic solvents were not formed; instead its conversion into xantho-ribalinium, was proved by UV absorption (Cfr. with Fig. 1) and recovery of the starting material (by acidification, precipitation as reineckate and transformation into the chloride with Dowex-2 chloride).

(b) The yellow colour of an aqueous solution of II disappeared upon acidification; subsequent addition of magnesium perchlorate gave a crystalline salt identified as ribalinium perchlorate[1] by m.p. and mixed m.p. By means of a column of Dowex-3 hydrochloride and MeOH as solvent, the same reconversion was attained; the eluted chloride of Ia was identified by paper chromatographic mobility,[1] m.p. and admixture m.p. and IR spectra.

Ring-opening of O^6*-methylribalinium* (Id)

Iodide of Id[1] (0·001 mole) was dissolved with magnetic stirring in 50 ml of borax–sodium carbonate buffer pH 10·6 and the colourless solution was left 24 hr at room temp. Extraction with $CHCl_3$ gave an enantiomeric mixture of III (0·282 g; 88%), $[\alpha]_D^{10°}$ −12° (*c*, 0·80); its IR ($CHCl_3$) was identical with that of the laevo or racemic form isolated as follows. Fractionation from ethyl acetate (1·4 ml) provided 0·134 g of O^6*-methylribalone* (III), m.p. 117–118°, raised by further recrystallizations to 119–120°, which showed no optical rotation. The UV (neutral or acid medium), IR ($CHCl_3$) and NMR (60 Mc, in $CDCl_3$) spectra were identical with those of the laevo isomer described below, while very slight differences in the solid phase IR spectra (Nujol) were noted. (Found: C, 63·42; H, 7·27; N, 4·63; O, 24·67; OCH_3, 19·16; N—CH_3, 5·44; active H, 0·56. $C_{17}H_{23}NO_5$ requires: C, 63·53; H, 7·22; N, 4·36; O, 24·89; two OCH_3, 19·31; one N—CH_3, 4·67; two active H, 0·63%.)

The mother liquor from the above fractionation was filtered through a column of 1·5 g of alumina (Woelm, neutral, activity V) using ethyl acetate; the eluted material was crystallized from ethyl acetate–cyclohexane (1:4) yielding 0·106 g of *laevo*-III, m.p. 101–102° unchanged by further purifications; $[\alpha]_D^{22°}$ −38° (*c*, 1·18); in neutral alcohol 50° and 96° or acid medium, λ_{max} 215 mμ (log ε 4·49), 234 (4·62), 276 (3·95), 283 infl. (3·88), 344 (3·88) λ_{min} 220 (4·47), 262 (3·78), 301 (3·04); ν_{max} ($CHCl_3$; in 1600 cm^{-1} region) 1632 (sh), 1616, 1580, 1503, 1467 cm^{-1}. (Found: C, 63·44; H, 7·07; N, 4·56; O, 24·97; 23 H by NMR; mol.wt. 327 (Rast). $C_{17}H_{23}NO_5$ requires: C, 63·53; H, 7·22; N, 4·36; O, 24·89%; mol.wt. 321.)

Solution of 20·0 mg (−)O^6-methylribalone (III) in 0·2 ml MeOH and 1·5 ml buffer pH 10·6 exhibited $[\alpha]_D^{20°}$ −58° which remained unchanged after 6 days at room temp; the material recovered by chloroform extraction was identical (m.p., IR spectrum and optical rotation in MeOH) with the starting material, thus showing that under the conditions used for cleavage no racemization occurs.

Degradative oxidation of O^6*-methylribalone* (III)

Solutions of III (0·0002 mole) in 8 ml MeOH–EtOH (1:5) and of sodium periodate (0·0006 mole) in 8 ml water were mixed at room temp and left for 0·5 hr under N_2. The solution was then distilled (N_2 current) collecting 4 ml into a saturated solution (10 ml) of 2,4-dinitrophenylhydrazine in 2N HCl; the precipitate was filtered off and crystallized from alcohol furnishing 18 mg of acetone 2,4-dinitrophenylhydrazone, m.p. 120–121°, identified by mixed m.p. and IR absorption comparison (KBr) with an authentic sample.

The undistilled fraction was cooled under N_2 and extracted with CH_2Cl_2; the extract was added to 12 ml of a saturated alcoholic solution (acidified with conc. HCl) of 2,4-dinitrophenylhydrazine. The CH_2Cl_2 was removed under red. press. and the precipitate recrystallized from toluene giving 32 mg of the 2,4-*dinitrophenylhydrazone of* 1-*methyl*-3-(*β-oxoethyl*)-4,6-*dimethoxy*-2-*quinolone*, m.p. 210–212° (dec). (Found: C, 54·63; H, 4·40; N, 15·41; O, 24·77. $C_{20}H_{19}N_5O_7$ requires: C, 54·42; H, 4·34; N, 15·87; O, 25·37%.)

Isolation of the intermediate epoxide (IV)

N,N-Dimethylformamide was kept several days over KOH then decanted, rectified and stored over CaH_2. After distillation (ca. 8 ml) directly into a flask containing O^6-methylribalinium (Id)[1] iodide (0·001 mole: dried at 56°/high vac). and protected from humidity, by magnetical stirring at ambient temp a clear solution was obtained. An oil dispersion of NaH (0·0009 mole) was added and the stirring continued until disappearance of the latter. The solution was maintained 10 hr at 56°

(or 24 hr at 20°) and then evaporated to dryness *in vacuo*. The residue was heated with 10 ml hexane and the supernatant phase decanted at room temp; this extraction was repeated 5 times.

The hexane was distilled off from the combined extracts and the residue crystallized from cyclohexane affording 64 mg of the *epoxide* (IV), m.p. 108–109°, optically inactive in MeOH at room temp in the range 600–380 mμ;[17,18] in neutral or acid medium (the absorption in acid medium remained almost unchanged after 5 days at room temp) λ_{max} 222 mμ (log ε 4·65), 246 (4·61), 287 (4·00), 296 (3·99), 330 (3·87), 340 (3·88), λ_{min} 235 (4·57), 263 (3·66), 292 (3·98), 311 (3·65), 335 (3·87); ν_{max} (in 1600 cm^{-1} region) 1645, 1628, 1580, 1507 cm^{-1}. (Found: C, 67·29; H, 7·01; N, 4·81; O, 21·14. $C_{17}H_{21}NO_4$ requires; C, 67·31; H, 6·98; N, 4·62; O, 21·10%.)

The appearance in the NMR spectrum of two well-separated lines corresponding to the non-equivalent methyl groups (in $CDCl_3$, δ 1·31 and 1·45 ppm) proved very useful for detection and purity determinations during the isolation; the accompanying substance III, formed by the presence of traces of water, shows a single sharp peak of two methyls (6 H) at δ 1·32 ppm.

Cleavage of the epoxide (IV)

Solution of the epoxide (5 mg) in a mixture of 0·1 ml MeOH and 0·2 ml buffer pH 10·6 (borax–sodium carbonate) was maintained at 20° for 2 hr. Absorption in the UV measured in acid medium indicated that the conversion to O^6-methylribalone (III) was complete.

The reaction mixture was extracted with chloroform which was then dried ($MgSO_4$) and evaporated to dryness. Crystallization of the residue from ethyl acetate gave 3 mg of racemic-III, m.p. 118–119·5°, identified by admixture m.p. and IR spectra.

Ring-opening of ribalinium (Ia) *chloride*

A mixture of Ia chloride (0·001 mole) and aqueous 1N NaOH (3 ml) was allowed to stand at room temp for 3 days. Before complete dissolution of the ribalinium salt, yellow crystals of xantho-ribalinium (II) begun to separate copiously but soon were redissolved resulting a clear yellow solution.

By addition of NH_4Cl (0·0045 mole) and after cooling, 0·299 g (97%) of a mixture of (+) and (−) *ribalone* (V), $[\alpha]_D^{19°}$ −24° (*c*, 0·99) crystallized; its IR spectrum in bromoform solution was identical with those of the following racemic or laevo compounds.

The enantiomeric mixture was refluxed 15 min with ethyl acetate (6 ml) and after being left 3 hr at room temp was centrifuged to separate the solid and liquid phases.

The former compound (0·190 g, m.p. 182–188°) was recrystallized from absolute EtOH to constant m.p. 198–200°; optically inactive; the IR ($CHBr_3$) and UV absorptions were identical with those of the laevo isomer described below. (Found: C, 62·41; H, 6·63; N, 4·70; O, 25·69. $C_{16}H_{21}NO_5$ requires: C, 62·52; H, 6·88; N, 4·56; O, 26·03%.)

Removal of the solvent from the liquid phase afforded a residue (0·105 g, m.p. 176–178°) which was repeatedly crystallized from acetone to constant m.p. 184–186°; $[\alpha]_D^{16°}$ −38° (*c*, 1·02); in neutral or acid medium λ_{max} 213 mμ (log ε 4·57), 234 (4·67), 277 (4·05), 284 infl. (3·97), 347 (3·93) λ_{min} 220 (4·53), 262 (3·87), 302 (3·16); ν_{max} (in 1600 cm^{-1} region) 1620, 1584, 1549, 1505; ν_{max} ($CHBr_3$) 1620, 1580, 1503 cm^{-1}. The Nujol peak at 1549 cm^{-1} is the strongest one and is absent in the Nujol spectrum of racemic ribalone. (Found: N, 4·84; $C_{16}H_{21}NO_5$ requires: N, 4·56%.)

Methylation of ribalone (V) *to* O^6*-methylribalone* (III)

To a solution of (−) ribalone (V) (24 mg) in MeOH (0·5 ml) powdered anhydrous K_2CO_3 (12 mg) was added and the mixture magnetically stirred for 15 min; after addition of MeI (0·15 ml, excess) the mixture was stirred at 45° (Teflon-stoppered tube) during 12 hr. Removal of the solvent *in vacuo* left a residue which was partitioned between aqueous 1N NaOH (1 ml) and chloroform (3 ml); the organic phase was washed with water, dried with $MgSO_4$ and evaporated to dryness.

The solution of the residue in ethyl acetate (4 ml) was filtered through a column of alumina Woelm, neutral, activity V (0·160 g); the eluate was evaporated under red. press. and the remaining material crystallized from ethyl acetate–cyclohexane providing (−)O^6-methylribalone (III) (19 mg; 76% yield), m.p. 101–102°. $[\alpha]_D^{15°}$ −38° (*c*, 1·00). It was identified by mixed m.p. and by comparison of the IR spectra with the sample obtained above by ring-opening of Id iodide.

[17] We are indebted to Prof. C. Djerassi (Stanford University, U.S.A.) for this measurement.

[18] Two other samples coming from different experiments were also inactive using MeOH or N,N-dimethylformamide as solvents (*c*, 1·0; 25°; Na light).