Rutacridone-epoxide, a New Acridone Alkaloid from *Ruta graveolens**

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Rutacridone-epoxide was isolated from roots and callus tissue cultures of *Ruta graveolens L.* and identified by ^{1}H-NMR and ^{13}C-NMR methods.

Several acridone alkaloids have been isolated from roots of *Ruta graveolens* (for summary see [1, 2]). One of the main alkaloids is rutacridone (**1**) which seems to be a precursor of several derivatives with hydroxyl or chlorine functions at the isopropyl moiety. As an intermediate an 18,19-epoxide of rutacridone could be postulated [1, 3] that has not yet been identified.

Screening for antimicrobial activity of CH_2Cl_2 extracts of both, roots of *R. graveolens* and tissue cultures made from shoots of *R. graveolens*, showed a yellow zone of distinct antimicrobial activity against the organisms used (*Bacillus subtilis, Serratia marcescens, Mycobacterium phlei*) when tested by TLC-methods published by Wolters [4]. The substance was isolated and purified by column chromatography on silica gel and subsequent preparative layer chromatography using its antimicrobial activity for detection.

The UV spectrum of the substance was similar to those of acridone alkaloids [5] exhibiting maxima at $\lambda = 390$ nm ($\log \varepsilon = 3.08$), 329 (3.32), 300 (3.57), 273 (3.95), 263 (3.83), 249 (3.80), 226 (3.57) in EtOEt and thus it is very similar to rutacridone [6]. After addition of methanolic $FeCl_3$ reagent a colour change from yellow to green was observed and the substance showed no fluorescence in MeOH; both observations are indicative of a free C-5-OH [5]. The mass spectrum of the compound showed an M^+ at *m/e* 323 (60%) which is 16 daltons greater than rutacridone. Further main fragments are observed at *m/e* 307 (13%), 292 (100%), 277 (35%) and 265 (25%).

The ^{1}H-NMR again is very similar to that published for rutacridone ([7], see Table I). In the present case spectral simulation and the high field (400 MHz) spectra allowed determination of all the relevant coupling constants (Table I). Differences in the spectra of rutacridone and the new compound are seen in the loss of the resonance lines of the two methylene protons attached at C-19 at $\delta = \sim 5$ ppm [7] and the appearance of two new doublets centered at 2.98 and 2.70 ppm which are couplet with each other (proved by homonuclear decoupling) with a coupling constant of 4.65 Hz. This is in agreement with a geminal coupling of two non equivalent protons of an oxiran grouping [8] and is strongly indicative of an 18,19-epoxide of rutacridone. Comparison of the proton decoupled ^{13}C-NMR of the new compound with that of rutacridone ([9], see Table II), again shows great similarities with the exception of C-18 and C-19, which resonate at 57.50 ppm and at 50.98 ppm respectively, and are consistent with an 18,19-epoxide [10].

Nuclear Overhauser Enhancement difference spectra at 400 MHz showed unambiguous enhancements for H-4 and for H-8 and H-9 (Table I) upon irradiation of the methyl protons of the N-CH_3 group. This gives direct proof of the angular annelation of the dihydrofuran moiety which has only being previously determined by indirect interpretation of NOE experiments on rutacridone [11]. Although the configuration of the chiral center at C-2 and the new one at C-18 is not yet established the data verify that the new substance is 1,2-dihydro-5-hydroxy-11-methyl-

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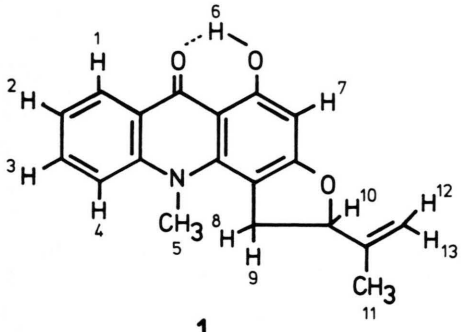
Table I. Proton NMR data for rutacridone (**1**) [a] and its epoxide (**2**) (δ-scale, TMS = 0.0 ppm).



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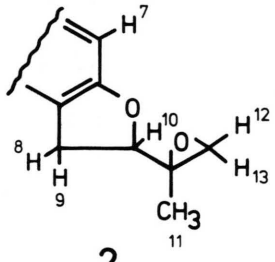


2

	Shifts (multiplicity) [b] [ppm]		Coupling constants [Hz]	
	1	**2**	**2**	
H-1	8.20 (d)	8.36 (d, d)	(1 – 2)	8.02
H-2	7.35	7.26 (m)	(1 – 3)	1.73
H-3		7.70 (m)	(1 – 4)	0.48
H-4		7.36 (m)	(2 – 3)	7.06
H-5	3.85 (s)	3.93 (s)	(2 – 4)	0.77
H-6	15.20	15.17 (s)	(3 – 4)	8.70
H-7	6.20 (s)	6.22 (s)	(8 – 9)	14.25
H-8	3.40 (d)	3.53 (d, d)	(8 – 10)	7.64
H-9		3.74 (d, d)	(9 – 10)	9.27
H-10	~ 5.40	4.76 (d, d)	(11 – 12)	0.47
H-11	1.75 (s)	1.43 (b)		
H-12	~ 5	2.98 (d, q)		
H-13		2.70 (d)		

[a] Data for rutacridone are taken from reference [7] and [15].
[b] s = singlet, d = doublet, q = quartet, b = broadened.

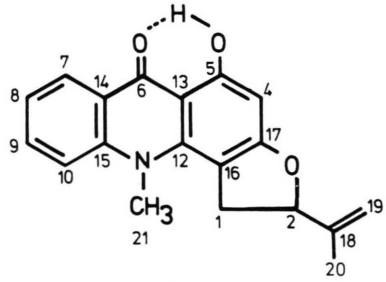
2-(1-methylepoxyethyl)furo[2,3-c]acridin-6(11 *H*)-one (rutacridon-epoxide, **2**).

From a biogenetical point of view the new acridone alkaloid must be the hitherto missing link between rutacridone and its derivatives, which are hydroxylated at C-18, C-19 and C-20 (sometimes followed by glucosylation or esterification) and in some cases are additionally chlorinated at C-19 or C-20 [1, 3, 7, 14]. Rutacridone-epoxide appears at rather high concentration in the roots of *R. graveolens* and in minor amounts in the tissue cultures. Extracts of the herb possess an identical antimicrobial zone on TLC indicating that the epoxide is also located in a small amount in the upper parts of the plant. On the other hand rutacridone itself seems not to be the main alkaloid in this plant material. This indicates that the plant material under investigation is obviously chemically different to that which has been used by Reisch and coworkers [1, 3, 7, 14]. It is not yet clear whether a genetically controlled chemical variation of the plant or an ecological influence is the reason for this difference. However, antimicrobial activity could either not be, or only slightly, detected in extracts of herb samples of *R. graveolens* obtained from the trade market (Wolters, unpublished). Studies in detail of the antimicrobial activity of rutacridone-epoxide will be published elsewhere.

Experimental

The plant material was collected from *R. graveolens* plants grown in the Botanical Garden of the University of Braunschweig. The material was freeze dried before extraction. The callus tissue culture was prepared by Reinhard [12] from stems of *R.* gra-

Table II. Carbon chemical shifts for rutacridone (**1**)[a] and its epoxide (**2**) (δ-scale, TMS = 0.0 ppm).



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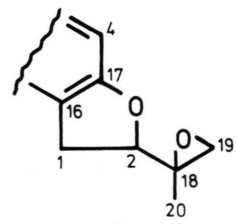

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Carbon	Chemical shifts, [ppm][b]		(Multiplicity)[c]
	1	**2**	
1	37.6	34.21	(t)
2	85.6	84.65	(d)
4	91.6	92.70	(d)
5	165.3 *	166.54 *	(s)
6	180.0	181.05	(s)
7	125.3	126.55	(d)
8	121.6	121.68	(d)
9	134.3	133.92	(d)
10	115.8	114.66	(d)
12	143.1 +	143.58 +	(s)
13	105.3	106.49	(s)
14	120.0	121.47	(s)
15	142.2 +	142.79 +	(s)
16	100.7	99.19	(s)
17	166.8 *	167.30 *	(s)
18	143.4	57.50	(s)
19	112.4	50.98	(t)
20	16.9	17.39	(q)
21	35.9	38.05	(q)

[a] Data for rutacridone are taken from reference [9].
[b] The assignment of the sets of resonances marked * and + are interchangeable within the set, as these cannot be assigned unambiguously from the data given in reference [9].
[c] Multiplicity in SFORD spectrum; s = singlet, d = doublet, t = triplet and q = quartet.

veolens and kindly provided by Prof. Czygan (Würzburg). The callus was grown in light and freeze dried before extraction. The dry material (16 g root material, 120 g callus tissue) was extracted with CH_2Cl_2 for two hours using an Ultra Turrax (Jahnke & Kunkel, Staufen) with subsequent Soxhlet extraction for another 6 h. The combined extracts were concentrated and chromatographed on SiO_2 in a column system (2.5 × 10 cm for the root extract, 6 × 30 cm for the callus extract) with Bz/EtOAc 60:40 as the solvent. The substance was eluated within the second column volume and detected by TLC using its antimicrobial activity [4]. The concentrated fractions were further purified by preparative layer chromatography on SiO_2 (Bz/EtOAc 60:40, $R_f \sim 0.3$) and following TLC in SiO_2 (Toluene/HCO_2Et/MeOH 7:2:1, R_f: 0.45). As a result ~ 7 mg have been obtained from callus tissue and ~ 8 mg from the roots. In some cases the UV-absorption and the reaction with $FeCl_3$ reagent [13] was used or detection on TLC. The UV spectrum was recorded in EtOEt using a Shimadzu UV-200-S spectrophotometer. The NMR spectra have been recorded at ambient temperature in $CDCl_3$ on a Varian XL-100-12 and a Bruker WM-400 in the Fourier transform mode with TMS as an internal standard.

Acknowledgement

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