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Semisynthetic Derivatives of Madurahydroxylactone and Their Antibacterial Activities

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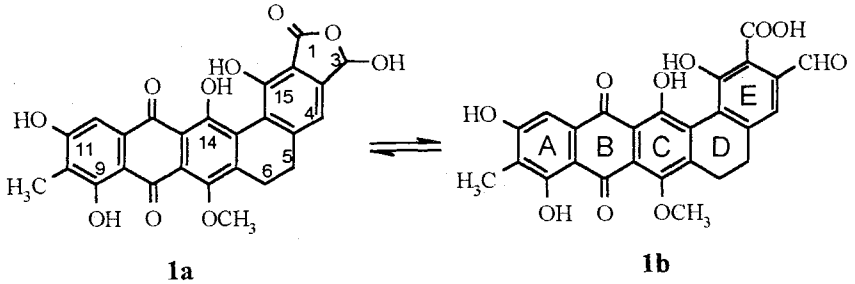
Madurahydroxylactone is a secondary metabolite from *Nonomuria rubra* (former *Actinomadura rubra*) with *in vitro* activity against Gram-positive bacteria and belongs to the family of benzo[*a*]naphthacenequinones. A series of derivatives of madurahydroxylactone were synthesized to investigate the effect on the antibacterial activity. Reaction with alcohols and amines gave cyclic acetals or aminals derived from the lactone form, whereas other amino reagents like hydroxylamines and acyl or sulfonyl hydrazides led to the corresponding imine derivatives of the aldehyde. Hydrazine, alkyl and aryl hydrazines react with madurahydroxylactone under cyclization to give compounds of the new heterocyclic basic structure naphthaceno[1,2-*g*]phthalazine. Some new compounds strongly inhibit Gram-positive bacteria, in part stronger than the parent compound.

In the seventies, in a search for pigment-forming actinomycetes, a strain of *Nonomuria rubra* (formerly designated *Actinomadua rubra*) was isolated which produces a mixture of red coloured secondary metabolites possessing indicator properties and showing activity against Gram-positive bacteria[1,2]. This complex, formerly called maduramycin, consists of madurahydroxylactone and homologues thereof. Recently single crystal X-ray structure analysis established madurahydroxylactone as 3,9,11,14,15-pentahydroxy-7-methoxy-10-methyl-1,8,13-trioxo-1,3,5,6,8,13-hexahydronaphthaceno[1,2-*f*]isobenzofurane **1a**[3,4]. Thus madurahydroxylactone is the prime component of the novel group of benzo[*a*]naphthacenequinone antibiotics, famous members of which are the antifungal benanomicins[5] and pradimicins[6]. Madurahydroxylactone is a weak acid and in acidic or neutral medium it forms a stable lactone, insoluble in water (**1a**)[7]. In alkaline medium, however, madurahydroxylactone readily dissolves forming alkali salts of an *ortho*-formylcarboxylic acid called maduranic acid (**1b**, 3-formyl-1,9,11,14-tetrahydroxy-7-methoxy-10-methyl-8,13-dioxo-5,6,8,13-tetrahydrobenzo[*a*]naphthacene-2-carboxylic acid). Analogous

Fig. 1.



1a **1b**

behaviour was observed for phthalaldehydic[8].

As madurahydroxylactone **1a** exhibited an interesting but therapeutical insufficient antibacterial activity we started a derivatization programme. This report presents the syntheses and antibacterial activities of derivatives.

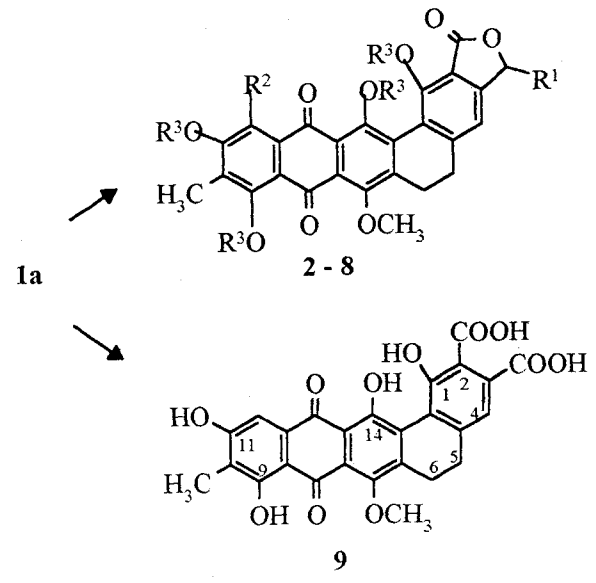
Chemistry

Derivatives based on the hydroxylactone moiety **1a** (Table 1): The backbone of madurahydroxylactone is a benzo[*a*]naphthacenequinone. The rings C and E constitute a 2,2′-dihydroxy-biphenyl moiety in which the phenyl moieties are further bridged by an ethano linker (C5~C6 in ring D). Whereas in α,α-disubstitued biphenyls without ethano bridge noncoplanarity is enforced, the X-ray structure of madurahydroxylactone shows only a moderate torsion in the C~E-biphenyl[3]. The situation changes in the case of substitution of the corresponding phenolic hydroxyls which are directed towards each other. Completely acetylated derivatives of madurahydroxylactone and related compounds are not easily achieved and the esters are relatively unstable under hydrolytic conditions. Thus, the ^{1}H NMR spectra of the pentaacetyl derivative **2**, obtained by reaction of madurahydroxylactone **1a** with acetic anhydride in pyridine, clearly showed the presence of two diastereoisomers at room temperature. This is a consequence of the fact that the pentaacetate **2** contains a stereogenic center at C3 and a chiral axis from ring C to E. INEPT experiments ($CDCl_3$, 300 K) allowed to assign the singlets at 7.37 and 7.40 ppm to H-3 and the singlets at 7.41 and 7.45 ppm to H-4. When the temperature was raised the frozen ethano bridge began to flip and spectra obtained at 380 K (DMSO-d_6) showed only one signal for the protons at C-3 (7.47 ppm) and C-4 (7.71 ppm), respectively. Although the protons of the ethano bridge exhibited a similar behaviour, full interpretation of the complex signals, obtained at room temperature was not possible.

We found that madurahydroxylactone **1a** forms a well crystalline derivative **3** upon heating with urea in acidic solution. The urea derivative is insoluble in tetrahydrofuran and can be used for purification of crude madurahydroxylactone[4].

Lower alkyl homologues of madurahydroxylactone had been claimed to be isolated from the culture medium of a mutant of *Actinomadura rubra*[2]. We prepared a series of corresponding 3-*O*-alkyl derivatives (**4a~f**) by boiling madurahydroxylactone in a mixture of tetrahydrofuran and an excess of the desired alcohol. The reaction can be accelerated by addition of a catalytic amount of concentrated sulfuric acid in the presence of molecular sieves to bind the reaction water. Specimens of synthetic and natural compounds showed identical spectroscopic and physico-chemical properties thus confirming the proposed structures. The more nucleophilic aliphatic and aromatic amines readily reacted with madurahydroxylactone to form the 3-amino derivatives **5a~c** (from primary amines) and **6a**, **b** (from secondary amines) even in the absence of any catalyst.

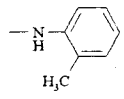
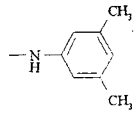
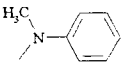
Fig. 2.



It was observed that prolonged treatment of madurahydroxylactone in alkali caused decomposition of the natural compound. Reaction in concentrated potassium hydroxide under controlled conditions led to two distinct products which could be unequivocally characterized as the dicarboxylic acid **9** and the maduralactone **7**. Obviously, the *ortho*-formylcarboxylic acid moiety in madurahydroxylactone underwent a Cannizzaro reaction. In the presence of formaldehyde, a crossed Cannizzaro reaction occurred with concomitant introduction of a further methyl substituent at C12 in ring A leading to 12-methylmaduralactone **8**.

Derivatives based on the *ortho*-formylcarboxylic acid moiety of **1b** (Fig 3, Table 2 and 3): Hydrazides of both carboxylic and sulfonic acids as well as *O*-alkyl hydroxylamines reacted with madurahydroxylactone **1** in boiling acetic acid to yield the corresponding hydrazones and oximes of the aldehyde (**10~14**). Exhaustive methylation of the methoxime **14** leading to the tetramethyl derivative **15** was accomplished with sodium hydride and dimethylsulfate in polar aprotic solvents like *N,N*-

Table 1. Data for compounds **2**~**8** (See Fig. 2).

Compound	R^1	R^2	R^3	UV λ_{max}^{MeOH} (ε)	Yield (%)	FAB-MS $(M+H)^+$
2	$OCOCH_3$	H	$COCH_3$	293 (8500) 364 (3900)	78	701
3	$NHCONH_2$	H	H	295 (8900) 467 (6500)	74	533
4a	OCH_3	H	H	295 (6800) 465 (4400)	84	505
4b	OC_2H_5	H	H	292 (4700) 468 (2700)	95	519
4c	O-nC_3H_7	H	H	302 (12300) 466 (12200)	97	533
4d	O-isoC_3H_7	H	H	301 (13000) 465 (11700)	92	533
4e	O-nC_4H_9	H	H	293 (8000) 466 (4800)	100	547
4f	O-n$C_{12}H_{25}$	H	H	292 (6200) 496 (3900)	84	659
5a	NH-C_6H_5	H	H	283 (4100) 478 (1800)	70	566
5b		H	H	289 (5950) 478 (2750)	62	580
5c		H	H	290 (14500) 474 (6200)	66	594
6a	N-$(CH_3)_2$	H	H	n.d.	85	518
6b		H	H	291 (12200) 334 (9510)	70	580
7	H	H	H	297 (15800) 493 (10800)	9	476
8	H	CH_3	H	303 (14300) 472 (11800)	76	489

dimethylformamide.

Hydrazine as well as alkyl- or aryl-substituted hydrazines differ in their reaction from that of the hydrazides. With madurahydroxylactone **1a** in boiling acetic acid, heterocyclic compounds of the novel naphthaceno[1,2-*g*]phthalazine structure are formed (**16**~**21**). This reaction corresponds to the synthesis of phthalazin-1-ones from phthalaldehydic acid[9]. The 2-(2-acetoxyethyl)-derivative **19** was formed from **1** and 2-hydroxyethylhydrazine and subsequent acetylation by the acetic acid solvent.

The phenolic hydroxyl group in position 12 is the most reactive one. Under alkaline conditions, the phthalazinones (**18**, **21**) were selectively monoacylated in high yield as was demonstrated with methyl chloroformate (**24**, **25**). Forced acylation conditions, *e.g.* heating with acetic anhydride and pyridine, were necessary to prepare the fully acylated compounds **22** and **23**.

The tetramethyl derivative **28** was synthesized from compound **21** by reaction with an excess of sodium hydride and iodomethane in dimethylformamide. When the same reaction was carried out using equimolar amounts of iodomethane as to the four hydroxy groups a mixture of the di-, tri- and the above-mentioned tetramethylated derivatives **26**, **27** and **28**, were obtained.

Antimicrobial Activity

The *in vitro* antibacterial activities of the madura-hydroxylactone derivatives were determined by minimal

inhibitory concentrations (MIC's) using the micro broth dilution method according to the NCCLS-guidelines[10]. The Gram-positive strains *Bacillus subtilis* ATCC 6633, *Micrococcus flavus* ATCC 10240, *Sarcina lutea* 125A, *Staphylococcus aureus* SG 511, *Mycobacterium phlei* SG 346 and the Gram-negative strains *Proteus vulgaris* OX19, *Proteus morganii* SG 464, *Pseudomonas aeruginosa* NCTC 10701 and *Escherichia coli* SG 485 were used as test organisms. Results are shown in Table 4. With the exception of aminals (3-amino maduralactones) **4d**, **5a**, **6a** and **6b** the compounds were inactive against all tested Gram-negative bacteria; in Table 4 only the MIC's against *P. vulgaris* OX19 are listed. The trimethyl derivative **26**, the tetramethyl compounds **15** (not in Table 4) and **28** and also the dicarboxylic acid **9** were inactive against all tested Gram-negative and Gram-positive bacteria. The other compounds exhibit different activities against the Gram-positive bacteria used. The aminals **5a**~**c** and **6a**, **b** are highly active against the tested strains of *Bacillus subtillis* and all cocci. The 3-alkoxy derivatives **4a**~**c** are active to some lower extend than the aminals, whereby the compounds **4d**~**f** with higher alkoxy groups are less active against *B. subtilis*. Out of the hydrazones compounds **11** (salicyloylcarbazone) and **13** (ethoxycarbazone) and the oxime **14** show good activities against Gram-positive bacteria except *M. phlei*. The new maduraphthalazines especially the 2-methyl derivative **17** and acetoxyethyl derivative **19**, show higher or equal activities against all Gram-positive bacteria than madurahydroxylactone **1a**, except against *M. phlei*, where the activity equal or significantly lower.

Antifungal activity was checked in a standardized agar diffusion assay by using *Glomerella cingulata* and *Sporobolomyces salmonicolor* 549 as test organisms. No antifungal activities were found among the compounds tested. Compounds with MIC <1 mg/ml against *S. aureus* SG 511 have to be further evaluated for their *in vitro* activity against different clonal types and multiresistent strains of epidemic *S. aureus* as well as for their *in vivo* activity. The most active compounds against *M. phlei* SG

Fig. 3.

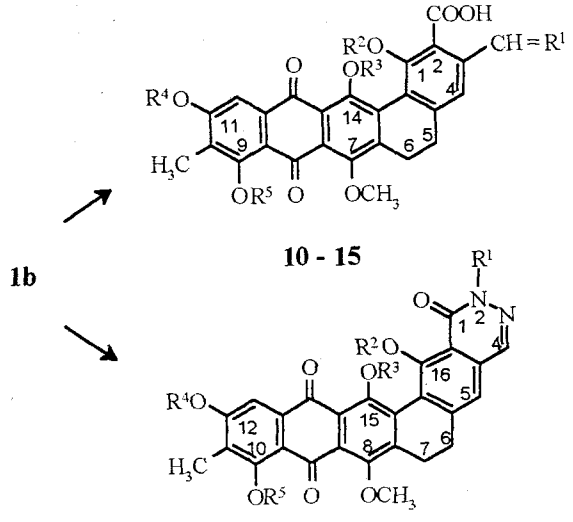
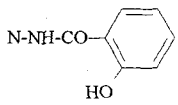
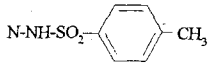


Table 2. Data for compounds **10**~**15** (See Fig. 3).

Compound	R^1	R^2-R^5	UV $\lambda_{max}^{solvent}$ (ε)	Yield %	FAB-MS $(M+H)^+$
10	N-NH-CO-C_6H_5	H	303 (18700)[x] 367 (18150)	73	609
11		H	289 (7450)[x] 476 (4030)	65	625
12		H	294 (10400)[x] 471 (6950)	70	659
13	N-NH-$COOC_2H_5$	H	308 (1300)[xx] 314 (12800)	69	577
14	N-OCH_3	H	313 (18100)[xx] 340 (17300)	33	520
15	N-OCH_3	CH_3	291 (10000)[x] 377 (2300)	76	590

[x] in MeOH [xx] in 0.01 M NaOH

Table 3. Data for maduraphthalazine derivatives **16**~**28** (See Fig. 3).

Compound	R^1	R^2	R^3	R^4	R^5	UV $\lambda_{max}^{solvent}$ (ε)	Yield %	FAB-MS $(M+H)^+$
16	H	H	H	H	H	335 (15880)[xx] 496 (16050)	75	487
17	CH_3	H	H	H	H	305 (7350)[xx] 497 (5000)	70	501
18	n-C_3H_7	H	H	H	H	304 (11100)[xx] 497 (12400)	75	529
19	$CH_2CH_2OCOCH_3$	H	H	H	H	300 (12350)[x] 471 (12400)	11	573
20	$CH_2CH(OC_2H_5)_2$	H	H	H	H	303 (13000)[x] 472 (11900)	80	645
21	C_6H_5	H	H	H	H	327 (15950)[xx] 498 (14550)	70	501
22	n-C_3H_7	$COCH_3$	$COCH_3$	$COCH_3$	$COCH_3$	307 (18000)[x]	65	697
23	C_6H_5	$COCH_3$	$COCH_3$	$COCH_3$	$COCH_3$	300 (13700)[x] 337 (10100)	73	731
24	n-C_3H_7	H	H	$COOCH_3$	H	291 (4750)[x] 477 (4500)	74	587
25	C_6H_5	H	H	$COOCH_3$	H	314 (2400)[x] 477 (2300)	74	621
26	C_6H_5	CH_3	H	CH_3	CH_3	292 (3700)* 442 (1700)	14	605
27	C_6H_5	H	H	CH_3	CH_3	n.d.	8	591
28	C_6H_5	CH_3	CH_3	CH_3	CH_3	306 (14800)[x] 358 (13500)	50	619

[x] in MeOH [xx] in 0.01 M NaOH

346 have to be checked for their activity against pathogenic mycobacteria including *M. tuberculosis* and *M. avium*.

Experimental

Melting points were determined with BOETIUS melting point apparatus and are uncorrected. UV spectra were recorded on a BECKMAN UV-spectrometer DU 640. ^1H and ^{13}C NMR spectra were recorded on a 300 MHz BRUKER NMR spectrometer. Interpretation of ^{13}C NMR spectra were confirmed by DEPT 135 experiments. Mass spectra were obtained with mass spectrometer AMD INTECTRA 402.

Urea-derivative **3**

To a solution of **1** (1 g, 2 mmol) in tetrahydrofuran (20 ml) and acetic acid (1 ml) was added urea (300 mg, 5 mmol) in water (2 ml) and the mixture was heated under reflux for 1 hour with stirring. After cooling to room temperature and standing for 2 hours the urea derivative was filtered off and washed with tetrahydrofuran and following with water removing the excess of urea to leave red crystals of **3**.

^1H NMR (DMSO-d_6) δ 2.10 (3H, s, 10-CH_3), 2.80 (2×2H, br s, 5-, 6-CH_2), 3.80 (3H, s, 7-OCH_3), 6.01 (2H, s, NH_2), 6.84 (1H, d, J=9.9 Hz, 3-CH), 7.29 and 7.11 (2×1H, s, 4-, 12-CH), 7.50 (1H, d, J=9.9 Hz, NHCO), 9.70, 11.24, 14.30, 13.59, (4×OH, s, 9-, 11-, 14-, 15-OH). ^{13}C NMR (DMSO-d_6) δ 8.20 (10-CH_3), 22.35, 29.74 (5-, 6-CH_2), 61.11 (7-OCH_3), 106.52 (3-C), 109.62, 112.83, 113.95, 118.40, 122.28, 129.24, 130.68, 146.47, 148.60, 149.56, 150.52, 153.75, 156.67, 157.26, 162.30, 162.30, 162.46, 167.37, 185.56, 187.65 (8-, 13-C).

Madurahydroxylactone Pentaacetate **2**

A solution of madurahydroxylactone (100 mg, 0.2 mmol) in a mixture of acetic anhydride (3 ml) and pyridine (1 ml)

Table 4. *In vitro* antibacterial activities of compounds **1**~**28** (MIC (μg/ml)).

Compound	*Bacillus subtilis* ATCC6633	*Micrococcus flavus* ATCC10240	*Sarcina lutea* 125A	*Staphylococcus aureus* SG 511	*Mycobacterium phlei* SG 346	*Proteus vulgaris* OX 19
1	0.2	0.8	0.8	1.6	1.6	100
2	25.0	12.5	125	12.5	50.0	100
3	3.2	50.0	50.0	50.0	50.0	100
4a	0.4	3.2	1.6	3.1	100.0	100
4b	0.8	1.6	1.6	3.1	100.0	100
4c	0.4	1.6	3.1	3.1	6.3	100
4d	6.3	3.2	3.1	3.1	3.1	25
4e	6.3	3.2	12.5	12.5	25.0	100
4f	1.6	25.0	25	12.5	100.0	100
5a	0.03	1.6	1.6	0.8	25.0	6.3
5b	0.05	0.4	1.6	0.8	50.0	100
5c	0.05	0.8	0.8	0.4	50.0	100
6a	0.4	0.8	0.8	0.2	50.0	25
6b	n.t.	1.6	3.1	0.8	12.5	25
7	6.3	1.6	3.1	3.2	50.0	>100
8	6.3	6.3	3.1	6.3	25.0	>100
9	100.0	50.0	25.0	50.0	50.0	100
10	25.0	50.0	50.0	25.0	50.0	100
11	0.4	0.8	1.6	0.8	100.0	>100
12	3.1	1.6	3.1	1.6	50.0	100
13	0.2	0.8	0.8	50.0	50.0	100
14	0.2	0.8	0.8	0.8	50.0	100
16	1.6	12.5	25.0	6.3	12.5	100
17	0.05	0.4	1.6	1.6	25.0	100
18	1.6	25.0	6.3	1.6	25.0	100
19	0.8	0.2	0.8	0.4	12.5	100
20	n.t.	0.8	12.5	0.4	25.0	>100
21	3.1	6.3	25.0	1.6	3.1	>100
22	3.1	0.4	1.6	6.3	1.6	100
23	25.0	12.5	25.0	12.5	6.3	>100
24	3.2	12.5	6.3	6.3	12.5	100
25	25.0	12.5	12.5	12.5	100.0	100
26	100.0	50.0	50.0	100.0	50.0	>100
27	6.3	3.1	25.0	0.8	50.0	100
28	>50.0	>50.0	>50.0	>50.0	>50.0	>100

was stirred at 50°C for 1 hour. Then the reaction mixture was diluted with ice water (10 ml) and stirred for 1 hour. The crystals were dissolved in chloroform and chromatographed on silica gel column eluting with chloroform. The fraction containing the desired product was concentrated and precipitated with acetonitrile to give yellow crystals, 110 mg (78%) (acetonitrile).

Anal Calcd for $C_{36}H_{28}O_{15}$: C 61.71, H 4.00.
Found: C 61.02, H 4.44.

^{1}H NMR (DMSO-d_6, T=300 K) δ 2.13 (3H, s, 10-CH_3), 2.09~2.41 (15H, s, 5×$COCH_3$), 2.47~2.49 (2×2H, m, 5-, 6-CH_2), 3.88 (3H, s, 7-OCH_3), 7.48 and 7.52 (1H, s, 3-CH), 7.78 and 7.80 (1H, s, 4-CH), 7.94 (1H, s, 10-CH).

^{1}H NMR (DMSO-d_6, T=380 K) δ 2.45 (4H, s, 2×CH_2), 7.79, 7.71, 7.47 (3H, s, 3×CH).

^{1}H NMR ($CDCl_3$) δ 1.45 (3H, s, 10-CH_3), 2.20~2.50 (15H, s, 5×$COCH_3$), 2.75~3.50 (4H, m, 2×CH_2), 3.90 (3H, s, 7-OCH_3), 7.37 and 7.40 (1H, s, 3-CH), 7.41 and 7.45 (1H, s, 4-CH), 7.81 (1H, s, 10-CH).

Madurahydroxylactone 3-Alkyl Ethers (**4a**~**f**)

Madurahydroxylactone (1.0 g, 2 mmol) and the cor-

responding alcohol (20 mmol) were dissolved in boiling dry tetrahydrofuran (30 ml) in the presence of molecular sieve (3Å, 2 g), conc. H_2SO_4 (2 drops) was added and the mixture was stirred under reflux for 2~3 hours until TLC ($CHCl_3$-MeOH-THF-H_2O, 80:20:10:2, v/v) indicated absence of any starting material. The hot mixture was filtered; in the case of **4a** and **4b**, the residue was extracted twice with boiling tetrahydrofurane. The filtrate was concentrated and the remaining solid was treated with ether, filtered off and dried *in vacuo*.

Madurahydroxylactone 3-Methyl Ether **4a**

^{1}H NMR (DMSO-d_6) δ 2.01 (3H, s, 10-CH_3), 2.79 (4H, br s, 5-, 6-H), 3.55, 3.87 (6H, 2s, 3-, 7-OCH_3), 6.44 (1H, s, 3-H), 7.12, 7.20 (4-, 12-H), 9.7 (1H, br s, OH), 11.18 (1H, br s, OH), 13.53 (1H, s, OH), 14.32 (1H, br s, OH).

^{13}C NMR (DMSO-d_6) δ 8.17 (10-CH_3), 22.30, 29.69 (5-, 6-C), 56.14, 61.05 (3-, 7-OCH_3), 101.39, 106.43, 113.51 (3-, 4-, 12-C), 109.56, 112.23, 113.82, 118.51, 120.53, 122.34, 128.89, 130.50, 146.73, 146.85, 150.14, 150.53, 153.78, 156.51, 162.16, 162.51, 166.52 (1-C), 185.39, 187.59 (8-, 13-C).

Madurahydroxylactone 3-Ethyl Ether **4b**

^{1}H NMR (DMSO-d_6) δ 1.26 (3H, t, J=7 Hz, 3-OCH_2CH_3), 2.02 (3H, s, 10-CH_3), 3.53 (4H, br m, 5-, 6-H), 3.79 (3H, s, 7-OCH_3), 3.88 (2H, q, J=7 Hz, 3-OCH_2CH_3), 6.50 (1H, s, 3-H), 7.12, 7.21 (2H, 2s, 4-, 12-H), 9.7 (1H, br s, OH), 11.18 (1H, s, OH), 13.54 (1H, s, OH), 14.32 (1H, br s, OH).

^{13}C NMR (DMSO-d_6) δ 8.16 (10-CH_3), 15.13 (OCH_2CH_3), 22.30, 29.67 (5-, 6-C), 61.04 (7-OCH_3), 64.97 (OCH_2CH_3), 100.58 (3-C), 106.41, 113.80 (4-, 12-C), 109.56, 112.14, 113.47, 118.50, 120.41, 122.30, 128.93, 130.51, 146.70, 147.22, 150.07, 150.53, 153.74, 156.52, 162.15, 162.50, 166.62 (1-C), 185.40, 187.60 (8-, 13-C).

Madurahydroxylactone 3-Propyl Ether **4c**

^{1}H NMR (DMSO-d_6) δ 0.94 (3H, t, J=7 Hz, 3-$OCH_2CH_2CH_3$), 1.65 (2H, qt, J=7 Hz, 3-$OCH_2CH_2CH_3$), 2.01 (3H, s, 10-CH_3), 3.52 (4H, br, 5-, 6-H), 3.68 (5H, m, 7-OCH_3, 3-$OCH_2CH_2CH_3$), 6.49 (1H, s, 3-H), 7.11, 7.20 (2H, 2×s, 4-, 12-H), 9.7 (1H, br s, OH), 11.17 (s, 1H, OH), 13.54 (1H, s, OH), 14.32 (1H, br s, OH).

^{13}C NMR (DMSO-d_6) δ 8.14 (10-CH_3), 10.39 (3′-CH_3), 22.28, 29.64 (5-, 6-C), 22.47 (2′-CH_2), 61.02 (7-OCH_3), 70.84 (OCH_2), 100.70 (C-3), 106.39, 113.78 (4-, 12-C), 109.53, 112.16, 113.42, 118.48, 120.41, 122.28, 128.90, 130.47, 146.68, 147.19, 150.07, 150.50, 153.73, 156.50, 162.13, 162.49, 166.59 (1-C), 185.37, 187.57 (8-, 13-C).

Madurahydroxylactone 3-Isopropyl Ether **4d**

^{1}H NMR (DMSO-d_6) δ 1.27, 1.32 (6H, 2 d, J=7 Hz, 3-$OCHCH_3$), 2.02 (3H, s, 10-CH_3), 3.72 (4H, br, 5-, 6-H), 3.80 (3H, s, 7-OCH_3), 4.22 (1H, sept, J=7 Hz, 3-$OCHCH_3$), 6.57 (1H, s, 3-H), 7.08, 7.21 (2H, 2s, 4-, 12-H), 9.6 (1H, br s, OH), 11.19 (1H, br s, OH), 13.55 (1H, s, OH), 14.31 (1H, br s, OH).

^{13}C NMR (DMSO-d_6) δ 8.19 (10-CH_3), 22.20, 29.66 (5-, 6-C), 23.18, 25.51 ($OCHCH_3$), 61.06 (3-OCH_3), 72.82 (OCH), 99.76 (3-C), 106.41, 113.43 (4-, 12-C), 109.58, 112.12, 113.80, 118.50, 120.27, 122.24, 128.98, 130.52, 146.68, 147.81, 150.00, 150.54, 153.67, 156.54, 162.15, 162.51, 166.77 (1-C), 185.42, 187.61 (8-, 13-C).

Madurahydroxylactone 3-Butyl Ether **4e**

^{1}H NMR (DMSO-d_6) δ 0.92 (3H, t, J=7 Hz, 3-$OCH_2CH_2CH_2CH_3$), 1.39 (2H, qt, J=7 Hz, 3-$OCH_2CH_2CH_2CH_3$), 1.62 (2H, t, J=7 Hz, OCH_2), 2.02 (10-CH_3), 3.43 (4H, br, 5-, 6-H), 3.79 (5H, m, 7-OCH_3, OCH_2), 6.48 (1H, s, 3-H), 7.10, 7.21 (2H, 2s, 4-, 12-H), 9.70 (1H, br s, OH), 11.17 (1H, s, OH), 13.54 (1H, s, OH), 14.31 (1H, br s, OH).

^{13}C NMR (DMSO-d_6) δ 8.14 (10-CH_3), 13.65 (4′-CH_3), 18.66 (CH_2), 22.26, 29.64 (5-, 6-C), 31.13 (CH_2), 61.02 (7-OCH_3), 68.93 (O-CH_2), 100.68 (3-C), 106.38, 112.14 (4-, 12-C), 109.54, 113.37, 113.78, 118.47, 120.49, 122.27, 128.91, 130.49, 146.67, 147.13, 150.05, 150.49, 153.73, 156.50, 162.13, 162.48, 166.58 (1-C), 185.38, 187.57 (8-, 13-C).

Madurahydroxylactone 3-Dodecyl Ether **4f**

After concentration of the filtrate, the residue was subjected to a silica gel column. Excess of reagent was separated by washing with chloroform. Elution with tetrahydofurane gave pure **4f**.

^{13}C NMR (DMSO-d_6) δ 8.04 (10-CH_3), 13.98 (12′-CH_3), 10.88, 29.14, 31.49 (CH_2), 60.24 (7-OCH_3), 68.67 (O-CH_2), 99.76 (3-C), 106.00, 112.48 (4-, 12-C), 109.65, 115.46, 116.55, 117.60, 123.33, 124.84, 133.42, 135.41, 143.52, 146.16, 147.34, 148.94, 156.54, 161.61, 162.07, 166.87 (1-C), 182.35, 186.62 (8,13-C).

3-Anilino-maduralactone **5a**

A solution of **1** (250 mg, 0.5 mmol) and aniline (95 mg, 1 mmol) in tetrahydofuran (5 ml) was refluxed for 30 minutes with stirring. The precipitated red crystals were washed with water and dried over P_4O_{10} to give **5a** (185 mg, 70%).

^{1}H NMR (DMSO-d_6) δ 2.07 (3H, s, 10-CH_3), 2.82 (2×2H, s, 5-, 6-CH_2), 3.81 (3H, s, 7-OCH_3), 6.62~7.82 (8H, s, 8×CH), 11.24, 13.57 (2H, s, 2×OH).

^{13}C NMR (DMSO-d_6) δ 13.30 (10-CH_3), 27.49, 33.80 (5-, 6-CH_2), 66.16 (7-OCH_3), 75.58, 111.43, 114.84, 119.00, 121.55, 123.45, 125.38, 127.76, 135.90, 136.78, 149.91, 151.73, 154.25, 155.73, 161.78, 161.97, 167.22, 167.54, 175.79, 190.72, 192.75 (8-, 13-C).

3-(2-Toluidino)-maduralactone **5b**

Prepared according to procedure of **5a** from **1** and *o*-toluidine.

Anal Calcd for $C_{33}H_{25}N_2O_9\times H_2O$: C 66.33, H 4.55, N 2.34.
Found: C 66.35, H 4.34, N 2.35.

^{1}H NMR (DMSO-d_6) δ 2.17, 2.08 (2×3H, s, 10-CH_3 and CH_3 of 2-toluidino), 2.82 (2×2H, s, 5-, 6-CH_2), 3.82 (3H, s, 7-OCH_3), 6.79~755 (7H, s, 7×CH), 9.61, 11.24, 13.60, 14.20 (4H, s, 9-, 11-, 14-, 15-OH).

3-(3,5-Dimethylanilino)-maduralactone **5c**

Prepared according to procedure of **5a** from **1** and 3,5-dimethylaniline.

Anal Calcd for $C_{33}H_{25}N_2O_9\times H_2O$: C 66.78, H 4.45, N 2.29.
Found: C 66.89, H 4.42, N 2.29.

^{1}H NMR (DMSO-d_6) δ 2.09 (3H, s, 10-CH_3), 2.21 (2×3H, s, 3′-, 5′-CH), 2.82 (2×2H, s, 5-, 6-CH_2), 3.82 (3H, s, 7- OCH_3), 6.46, 6.57 (3H, s, 3×CH), 7.01 (1H, s, 3-CH), 7.32 and 7.17 (2H, s, 4-CH and 12-CH), 9.71, 11.31, 13.60, 14.20 (4H, s, 9-, 11-, 14-, 15-OH).

3-Dimethylamino-maduralactone **6a**

To a solution of 500 mg (1 mmol) of madurahydroxylactone **1** in boiling tetrahydrofuran (20 ml) dimethylamine (5 ml) was added under heating to reflux. The mixture was heated for an additional 15 minutes. After cooling to room temperature the precipitant was filtered off, washed with tetrahydrofuran and with diethylether to leave 486 mg of the cyclic aminal.

^{1}H NMR (D_2O) δ 1.85 (3H, s, 10-CH_3), 2.4~2.7, (4H, m, 5-, 6-CH_2), 2.74, (6H, s, $N(CH_3)_2$), 3.50 (3H, s, 8-OCH_3), 6.77, 7.04, (2H, 2×s, 4-, 12-CH_2), 9.96 (1H, s, 3-CHON).

^{13}C NMR (D_2O) δ 8.59 (10-CH_3), 23.16, 29.26 (5-, 6-CH_2), 35.40 ($N(CH_3)_2$), 107.34, 112.813, 118.649, 118.833, 119.068, 125.754, 129.001, 131.020, 132.158, 133.309, 133.781, 142.109, 145.906, 148.117, 154.299, 162.033, 162.730, 174.541, 176.184, 185.412, 187.293, 195.662.

3-*N*-Methylanilino-maduralactone **6b**

Prepared according to procedure of **5a** from **1** and *N*-methylaniline.

^{1}H NMR (DMSO-d_6) δ 2.08 (3H, s, 10-CH_3), 2.62 (3H, s, N-CH_3), 2.82 (2×2H, s, 5-, 6-CH_2), 3.81 (3H, s, 7-OCH_3), 7.2~7.4 (7H, m, 7×CH), 9.81, 11.26, 13.60, 14.2 (4H, s, 9-, 11-, 14-, 15-OH).

Madurandicarboxylic acid **9** and maduralactone **7**

Madurahydroxylactone **1** (250 mg, 0.5 mmol) soluted in 10 M KOH (5 ml) was boiled with stirring for 2 hours. The mixture was diluted with water (10 ml) and carefully acidified under cooling with 5 M HCl (about 10 ml). The red crystals contained 39% **7** and 54% **9** according to HPLC (Eurospher® Si 100 7 C18 (acetonitrile - water, 3 : 2).

The crude product was stirred with saturated aqueous solution of $NaHCO_3$ and extracted with ethyl acetate. The aqueous phase was acidified with 2 M HCl. The collected product was purified by preparative thin layer chromatography (Si 60, chloroform - methanol, 10 : 1) to give 20 mg **9** (10%), mp 325°C (dec.).

^{1}H NMR (dioxan-d_8) δ 2.10 (3H, s, 10-CH_3), 3.17, 2.91 (2×2H, s, 5- and 6-CH_2), 3.82 (3H, s, 7-OCH_3), 7.18 and 7.13 (2×1H, s, 4-, 12-CH), 9.25, 9.65, 13.46, 14.85 (4H, s, 1-, 9-, 11-, 14-OH).

^{13}C NMR ($CDCl_3$) δ 187.17, 185.83 (8-, 13-C), 172.3, 167.95 (2-, 3- COOH), 60.97 (7-OCH_3), 30.7 and 25.5 (5-, 6-CH_2), 8.17 (10-CH_3).

The ethyl acetate phase was dried with Na_2SO_4 and concentrated. The product was precipitated with benzene. The crude material was purified by thin layer chromatography (Si 60, chloroform - methanol, 10 : 1 v/v) to give **7** (17 mg, 9%).

^{1}H NMR (DMSO-d_6) δ 2.07 (3H, s, 10-CH_3), 3.8 (3H, s, 7-OCH_3), 5.32 (2H, s, 3-CH_2O), 7.26 and 6.79 (2×1H, s, 4-, 12-CH), 11.14, 13.58, 13.84, 14.06 (4H, s, 4×OH).

^{13}C NMR (DMSO-d_6) δ 8.13 (10-CH_3), 30.27 and 22.07 (5-, 6-CH_2), 60.60 (7-OCH_3), 67.5 (3-CH_2O), 106.56, 107.90, 111.60, 114.89, 123.45, 134.13, 135.86, 147.23, 148.56, 149.24, 161.72, 180.69, 190.74 (8-,13-C).

12-Methyl-maduralactone **8**

To a mixture of madurahydroxylactone (purity 97%) (245 mg, 0.5 mmol) and aqueous formaldehyde (36%) (2 ml, 0.02 mol) in methanol (2 ml) was added KOH (2 g) with stirring. The mixture was refluxed for 1 hour. After cooling to ambient temperature the solution was diluted with water (10 ml) and carefully acidified under ice cooling with HCl to pH 3. The crude product was stirred in aqueous $NaHCO_3$ with stirring, filtered off, washed with water and dried to give red crystals of **8** (192 mg, 76%).

^{1}H NMR (DMSO-d_6) δ 2.10, 2.61 (2×3H, s, 10-, 12-CH_3), 2.77 (2×2H, s, 5-, 6-CH_2), 3.79 (3H, s, 7-OCH_3), 5.32 (2H, s, 3-CH_2), 7.09 (1H, s, 4-CH), 9.5, 10.2, 14.4, 14.5 (4H, s, 9-, 11-, 14-, 15-OH).

^{13}C NMR (DMSO-d_6) δ 9.18 (10-CH_3), 14.26 (12-CH_3), 29.91 and 22.32 (5-, 6-CH_2), 61.10 (7-OCH_3), 68.4 (3-CH_2O), 110.07, 111.31, 112.23, 114.87, 118.50, 118.56, 121.99, 123.68, 127.60, 129.07, 145.34, 149.73, 153.98, 155.84, 161.02, 161.53, 169.07, 185.82, 190.39 (8-, 13-C).

Maduranic Acid Benzhydrazone **10**

A solution of madurahydroxylactone (490 mg, 1 mmol) and benzhydrazide (140 mg, 1 mmol) in acetic acid (20 ml) were refluxed for 30 minutes. The crude product was dissolved in tetrahydrofuran, filtered and precipitated by addition of ligroin to give red crystals (450 mg, 73%), mp 268~270°C.

Anal Calcd for: C 65.13, H 3.97, N 4.60.
Found: C 64.63, H 4.30, N 5.05.

^{1}H NMR (DMSO-d_6) δ 2.07 (3H, s, 10-CH_3), 2.76, 2.81 (2×2H, s, 5-, 6-CH_2), 3.79 (3H, s, 7-OCH_3), 7.25~7.94 (7H, m, 7×CH), 8.62 (2H, s, CH=N and NHCO), 8.96, 11.14, 12.04, 13.57 (4×H, s, 4×OH).

^{13}C NMR (DMSO-d_6) δ 8.16 (10-CH_3), 22.49, 28.84 (5-, 6-CH_2), 61.01 (7-OCH_3), 104.61, 106.36, 109.676, 113.90, 115.67, 118.43, 119.12, 120.16, 122.19, 127.68, 128.38, 129.48, 130.63, 133.16, 134.04, 143.76, 146.71, 150.73, 155.04, 156.33, 162.08, 162.26, 162.44, 163.20, 170.13 (2-COOH), 187.7, 185.46 (8-, 13-C).

Maduranic Acid 2-Hydroxybenzhydrazone **11**

Prepared according to the procedure of **10** from **1** and 2-hydroxybenzhydrazide, red crystals, mp 262~266°C

Anal Calcd for $C_{33}H_{24}N_2O$: C 61.68, H 4.08, N 4.36.
Found: C 61.79, H 3.96, N 4.24.

^{1}H NMR (DMSO-d_6) δ 2.08 (3H, s, 10-CH_3), 2.80 (2×2H, s, 5-, 6-CH_2), 3.80 (3H, s, 7-OCH_3), 6.94 (2H, t, J=8.2 Hz, 2×CH), 7.28 (1H, s, CH), 7.44 (1H, t, J=7.7 Hz, CH), 7.49 (1H, s, CH), 7.90 (1H, d, J=7.8, CH), 8.83 (1H, s, CH=N), 11.17, 12.05, 13.56, 14.45 (4H, s, 1-, 9-, 11-, 14-OH), 11.86 (1H, s, 2-OH hydroxybenzhydrazone).

Maduranic Acid Tosylhydrazone **12**

Prepared according to the procedure of **10** from **1** and tosylhydrazide, red crystals, mp 303~305°C (dec.)

^{1}H NMR (DMSO-d_6) δ 2.15, 2.36, (2×3H, s, 2×CH_3), 2.71, 2.80 (2×2H, s, 5-, 6-CH_2), 3.78 (3H, s, 7-OCH_3), 7.18, 7.28 (2H, s, 4-, 12-CH), 7.41, 7.46, 7.78, 7.81 (4H, s, 4-CH of tosyl), 8.30 (1H, s, CH=N), 14.35 (br), 11.19, 11.65, 13.55 (4 H, s, 4×OH).

^{13}C NMR (DMSO-d_6) δ 8.12 (10-CH_3), 20.95 (CH_3 tosyl), 28.79, 22.37 (5- and 6-CH_2), 61.01 (7-OCH_3), 106.34, 109.67, 113.86, 115.52, 117.93, 118.42, 120.20, 122.20, 129.35, 127.14, 129.66 (*p*-phenylene), 130.65, 133.39, 136.21, 143.46, 144.04, 145.33, 146.56 (CH=N), 150.74, 155.37, 156.36, 162.07, 162.42, 170.05 (2-COOH), 187.7, 185.48 (8-, 13-C).

Maduranic Acid Ethoxycarbonylhydrazone **13**

Prepared according to the procedure of **10** from **1** and ethoxycarbonylhydrazide, red crystals, mp 303~305°C (dec.)

Anal Calcd for $C_{29}H_{24}N_2O_{11}$: C 60.42, H 4.20, N 4.86.
Found: C 59.91, H 4.30, N 4.86.

^{1}H NMR (DMSO-d_6) δ 1.24 (3H, t, OCH_2CH_3), 2.06 (3H, s, 10-CH_3), 2.78 (2×2H, s, 5-, 6-CH_2), 3.80 (3H, s, 7-OCH_3), 4.11~4.21 (2H, q, OCH_2CH_3), 7.37 and 7.28 (2×1H, s, 4-CH and 12-CH), 8.42 (1H, s, CH=N), 11.18 (br), 13.35, 13.58, 14.3 (4×OH, s, 1-, 9-, 11-, 14 -OH).

Maduranic Acid *O*-Methyloxime **14**

A solution of **1** (500 mg, 1 mmol) and *O*-methylhydroxylamine hydrochloride (200 mg, 1 mmol) in acetic acid (40 ml) was boiled under reflux 30 minutes to give of red crystals (172 mg (33%) of **14**, mp 267~270°C.

Anal Calcd for $C_{27}H_{21}NO_{10}\times CH_3COOH$:
C 60.10, H 4.35, N 2.70.
Found:
C 59.51, H 4.38, N 3.01.

^{1}H NMR (DMSO-d_6) δ 2.05 (3H, s, 10-CH_3), 3.91 and 3.79 (2×3H, s, 7-OCH_3 and CH=NO), 7.24 and 7.22 (2×1H, s, 4-, 12-CH), 8.54 (1H, s, CH=N), 13.58, 11.17 (2×H, s, 2×OH, other OH not shown).

^{13}C NMR (DMSO-d_6) δ 8.12 (10-CH_3), 22.35, 28.63 (5-, 6-CH_2), 60.95, 61.70 (7-OCH_3 and $NOCH_3$), 106.31, 109.63, 113.79, 115.97, 116.34, 118.34, 120.41, 122.19, 129.39, 130.65, 132.01, 144.73, 146.56, 148.15, 150.59, 156.60, 156.73, 162.05, 162.42, 170.12 (2-COOH), 187.59, 185.47 (8-, 13-C).

Red brown crystals in DMSO: for

Anal Calcd for $C_{27}H_{21}NO_{10}\times 2.5$ mol C_2H_6SO:
C 53.78, H 5.08, N 1.96, S 11.19.
Found:
C 53.16, H 4.84, N 2.07, S 11.16.

Tetramethyl Derivative of Maduranic Acid-*O*-methyloxime-methyl Ester **15**

To a cooled (0°C) solution of **14** (100 mg, 0.2 mmol) in dimethylformamide (5 ml) was added sodium hydride (150 mg, 6 mmol) in portions and after 5 minutes of iodomethane (2 ml, 30 mmol) were added. The mixture was heated with stirring to 50°C for 1 hour. After cooling the mixture was allowed to stay at ambient temperature overnight. To the brown mixture water and 0.1 M HCl were

added the yellow product was dissolved in tetrahydrofuran and precipitated with benzene to yield **15** (86 mg, 76%).

^{1}H NMR (CDC_3) δ 2.25 (3H, s, 10-CH_3), 3.60, 3.50 (2×3H, s, 2×OCH_3), 3.9~4.0 (5×3H, s, 5×OCH_3), 7.45, 7.47 (2H, s, 2×CH), 8.10 (1H, s, CH=N).

Maduraphthalazin-1-one **16**

A solution of madurahydroxylactone (80%) (293 mg, 0.2 mmol) and hydrazine hydrate (80%, 2 ml) in 10 ml acetic acid was refluxed with stirring for 30 minutes. The hot mixture was filtered, washed with water dried *in vacuo* over P_4O_{10} to give **16** (182 mg, 75%), mp >360°C.

Anal Calcd for $C_{26}H_{18}N_2O_8$: C 64.20, H 3.73, N 5.76.
Found: C 64.55, H 4.02, N 6.30.

^{1}H NMR (DMSO-d_6) δ 2.10 (3H, s, 11-CH_3), 2.77, 2.93 (2×2H, s, 6- and 7-CH_2), 3.83 (3H, s, 8-OCH_3), 7.25, 7.32 (2×1H, s, 5-CH and 13-CH), 8.40 (1H, s, 4-CH), 11.14, 13.09, 13.56, 14.06 (4H, s, 10-, 12-, 15-, 16-OH), 13.19 (1H, s, CONH).

^{13}C NMR (DMSO-d_6) δ 8.13 (12-CH_3), 22.28, 29.32 (6 and 7-CH_2), 61.01 (8-OCH_3), 106.26, 109.73, 111.48, 113.54, 114.02, 118.25, 119.09, 122.42, 128.64, 130.15, 130.75, 139.82, 140.64, 146.88, 148.62, 150.36, 157.33, 162.07, 162.40, 164.18, 185.59, 187.70 (9-, 14-C).

2-Methyl-maduraphthalazin-1-one **17**

A solution of madurahydroxylactone (80%, 293 mg, 0.2 mmol) and methylhydrazine (100 mg 0.05 ml, 1 mmol) in 5 ml of acetic acid was refluxed with stirring for 8 hours. The red crystals were filtered off, washed with water, and dried *in vacuo* over P_4O_{10} to give **17** (175 mg, 70%), mp >300°C (dec. 248°C). Further purification was accomplished by preparative thin layer chromatography (Merck plates Si 60, eluent: butyl acetate - acetic acid, 4 : 1 v/v).

^{1}H NMR (DMSO-d_6) δ 2.11 (3H, s, 11-CH_3), 2.52 (4H, s, 6- and 7-CH_2), 3.80, 3.78 (2×3H, s, 8-OCH_3 and 2-CH_3), 7.32, 7.12 (2×1H, s, 5-CH and 13-CH), 8.42 (1H, s, 4-CH), 11.19, 13.20, 13.55, 14.07 (4×1H, s, 10-, 12-, 15- and 16-OH).

^{13}C NMR (DMSO-d_6) δ 8.1 (11-CH_3), 29.23, 22.18 (6- and 7-CH_2), 60.96 (OCH_3), 106.22, 109.62, 111.19, 113.94, 118.14, 119.17, 122.31, 128.58, 129.72, 130.10, 130.65, 139.24, 146.82, 148.33, 148.57, 150.27, 156.71, 157.08, 157.29, 162.00, 162.32, 162.62, 164.13, 187.61, 185 (9-, 14-C).

2-*n*-Propyl-maduraphthalazin-1-one **18**

A solution of madurahydroxylactone (500 mg, 1 mmol) and *n*-propylhydrazine hydrochloride (120 mg 1 mmol) in 5 ml acetic acid was refluxed with stirring for 3 hours. The red crystals were filtered off, washed with water and vacuum dried over P_2O_5 to give **17** (400 mg, 75%), mp 350 ~351°C (dec.). Further purification was accomplished by preparative thin layer chromatography (Merck plates Si 60, eluent: butyl acetate - acetic acid, 4 : 1 v/v).

^{1}H NMR (DMSO-d_6) δ 0.90 (3H, t, J=7.3 Hz, $CH_2CH_2CH_3$), 1.78 (2H, tq, J=7.3 Hz, C–CH_2–C), 2.05 (3H, s, 11-CH_3), 3.85 (3H, s, 8-OCH_3), 4.08 (2H, m, N–CH_2), 7.32, 7.12 (2×1H, s, 5-CH and 13-CH), 8.48 (1H, s, 4-CH), 11.29, 13.40, 13.70, 14.07 (4×1H, s, 10-, 12-, 15- and 16-OH).

2-(2-Acetoxyethyl)-maduraphthalazin-1-one **19**

A solution of madurahydroxylactone (250 mg, 0.5 mmol) and 0.15 ml 2-hydroxy-ethylhydrazine in 30 ml acetic acid was boiled under reflux with stirring for 1 hour. The mixture was diluted with water, the crude product filtered off, dried and purified by preparative thin layer chromatography (Merck plates Si 60, eluent: butyl acetate - acetic acid, 4 : 1 v/v). The first zone (Rf=0.78) was isolated to give orange-red crystals of **19** (40 mg, 11%).

^{1}H NMR (DMSO-d_6) δ 1.95 (3H, s, 11-CH_3), 2.15 (3H, s, $OCOCH_3$), 3.90 (3H, s, 8-OCH_3), 4.45 (2H, s, –CH_2OCO–), 7.35, 7.25 (2×1H, s, 5-, 13-CH), 8.51 (1H, s, 4-CH), 11.22, 13.10, 13.61, 14.12 (4×H, s, 10-, 12-, 15-, 16-OH).

^{13}C NMR (DMSO-d_6), δ 8.2 (11-CH_3), 20.6 ($OCOCH_3$), 29.36, 22.31 (6-, 7-CH_2), 49.05 (NCH_2), 60.9 (OCH_2), 61.08 (8-OCH_3), 106.34, 109.74, 111.16, 113.60, 114.29, 118.29, 119.57, 122.51, 128.62, 129.65, 130.77, 139.79, 147.02, 148.83, 150.39, 156.96, 157.40, 162.40, 162.96, 170.36, 187.74, 185.63 (9-, 14-C).

2-(2-Diethoxy)ethyl-maduraphthalazin-1-one **20**

To a solution of 100 mg (0.2 mmol) of madurahydroxylactone **1** in of acetic acid (30 ml) were added 2,2-diethoxyethylhydrazine (300 μl). The mixture was heated to reflux. After 30 minutes the mixture was cooled. The solvent was evaporated to *ca.* a third of the original volume and treated with diethylether to precipitate the product. The red crystals were filtered off maintaining the desired product **20**, yield 104 mg (0.16 mmol, 80%).

^{1}H NMR (DMSO-d_6, T=320 K) δ 2.09, (3H, s, 11-CH_3), 2.50 (6H, t, J=7.5 Hz, OCH_2CH_3), 2.85 (4H, 2×br s, OCH_2CH_3), 3.33 (3H, s, 8-OCH_3), 4.25 (2H, d, J=7.5 Hz, NCH_2), 4.87 (1H, t, J=7.5 Hz, CHOO), 7.28 (1H, s), 7.33 (1H, s), 8.44 (1H, s, 4-H).

2-Phenyl-maduraphthalazin-1-one **21**

A solution of madurahydroxylactone **1** (250 mg, 0.2 mmol) and phenylhydrazine (110 mg, 1 mmol) in 5 ml acetic acid was refluxed with stirring for 2 hours. The red crystals were filtered off, washed with water, and dried *in vacuo* over P_4O_{10} to give **21** (198 mg, 70%), mp >300°C (dec). Further purification was accomplished by preparative thin layer chromatography (Merck plates Si 60, eluent: butyl acetate - acetic acid, 4 : 1 v/v).

^{1}H NMR (DMSO-d_6) δ 2.08 (3H, s, 11-CH_3), 3.81 (3H, s, 8-OCH_3), 7.27, 7.42 (2×1H, s, 5-, 13-CH), 7.48~7.65 (5H, m, 5-CH), 8.58 (1H, s, 4-CH), 11.15, 13.07, 13.56, 14.07 (4×H, s, 10-, 12-, 15-, 16-OH).

^{13}C NMR (DMSO-d_6) δ 8.17 (11-CH_3), 25.09, 29.35 (6-, 7-CH_2), 61.08 (8-OCH_3), 106.29, 109.76, 111.57, 113.62, 114.48, 118.28, 119.85, 122.56, 126.22, 128.19, 128.60, 128.71, 129.60, 130.78, 140.14, 140.61, 146.95, 149.13, 150.36, 157.35, 157.41, 162.11, 162.42, 185.52, 187.74 (9-, 14-C).

2-*n*-Propyl-10,12,15,16-tetraacetoxy-maduraphthalazin-1-one **22**

A solution of 2-*n*-propyl-maduraphthalazin-1-one **18** (100 mg, 0.2 mmol) in acetic anhydride (3 ml) and pyridine (1 ml) was warmed up to 100°C with stirring for 1 hour. The mixture was concentrated in vacuum and digested with water to give 90 mg (65%) yellow crystals of **22**.

^{1}H NMR (DMSO-d_6) δ 1.05 (3H, t, J=7.2 Hz, $NCH_2CH_2CH_3$), 1.86 (2H, q, J=7.2 Hz, $NCH_2CH_2CH_3$), 2.10 (3H, s, 11-CH_3), 2.20~2.60 (12H, s, 4×$COCH_3$), 3.90 (3H, s, 8-OCH_3), 4.15 (2H, s, NCH_2), 7.81, 7.80 (2H, s, 5-, 13-CH), 8.15 (1H, s, 4-CH).

^{13}C NMR ($CDCl_3$) δ 5.17 (3′-CH_3), 5.97 (11-CH_3), 15.6~16.6 (4×$COCH_3$), 16.61 (2′-CH_2), 24.87, 17.75 (6-, 7-CH_2), 47.05, 47.69 (NCH_2), 57.40 (OCH_3) 108.65, 116.17, 113.31 (5-, 13-CH), 126.26, 126.56, 126.83, 128.42, 131.13, 131.52, 133.68, 139.17 (4-CH), 142.02, 143.85, 148.39, 162.85, 163.84, 164.25, 170.04, 182.29, 182.60 (9-, 14-C).

2-Phenyl-10,12,15,16-tetraacetyl-maduraphthalazin-1-one **23**

A solution of 2-phenyl-maduraphthalazin-1-one **21** (250 mg, 0.5 mmol) in acetic anhydride (5 ml) and pyridine (1 ml) was warmed up to 50°C with stirring for 2 hours. The mixture was concentrated *in vacuo* and stirred with water to give yellow crystals of **23**.

Anal Calcd for $C_{32}H_{18}N_2O_8$: C 65.75, H 4.14, N 3.83.
Found: C 65.25, H 4.22, N 4.07.

FAB(+)-MS *m/z* 501.1 (M+1), Other peaks: 689 (−1 $COCH_3$), 647 (−2 $COCH_3$), 605 (−3 $COCH_3$).

^{1}H NMR ($CDCl_3$) δ 2.10 (3H, s, 11-CH_3), 2.36~2.49 (4×3H, s, 4×$COCH_3$), 3.86 (3H, s, 8-OCH_3), 7.51~7.53 (5H, m, phenyl), 7.94, 7.80 (2×1H, s, 5-, 13-CH), 8.56 (1H, s, 4-CH).

2-*n*-Propyl-12-methoxycarbonyl-maduraphthalazin-1-one **24**

To a solution of 2-*n*-propyl-maduraphthalazin-1-one **18** (264 mg, 0.5 mmol) in 5 ml 1 M sodium hydroxide at 0~5°C methyl chloroformate (0.5 ml) was added. The mixture was stirred at room temperature for 30 minutes and than extracted with chloroform. The organic phase was washed with water, dried and concentrated. The product was precipitated with benzene to give red crystals of **24**.

^{1}H NMR ($CDCl_3$) δ 0.91 (3H, t, J=7.3 Hz, $NCH_2CH_2CH_3$), 1.94 (2H, q, J=7.3 Hz, $NCH_2CH_2CH_3$), 2.10 (3H, s, 11-CH_3), 2.80, 3.05 (2×2H, s, 6-, 7-CH_2), 3.90 and 4.01 (2×3H, s, 8-OCH_3 and 12-$OCOOCH_3$), 7.05, 7.65 (2×1H, s, 5-, 13-CH), 8.15 (1H, s, 4-CH), 13.40, 13.61, 14.20 (3×1H, s, 10-, 15-, 16-OH).

^{13}C NMR ($CDCl_3$) δ 9.23 (CH_3), 11.14 (11-CH_3), 21.77, 22.76, 30.19 (6-, 7-CH_2, 2′-CH_2), 55.95 and 61.57 (8-OCH_3 and 12-$OCOOCH_3$), 52.25 (2-NCH_2), 112.25 and 113.36 (5-, 13-CH), 114.09, 114.35, 119.88, 127.18, 130.14, 130.14, 131.21, 138.85 (4-CH), 146.59, 148.33, 151.22, 153.02, 154.46, 158.17, 158.33, 162.83, 163.15, 187.04, 187.28 (9-, 14-C).

2-Phenyl-12-methoxycarbonyloxy-maduraphthalazin-1-one **25**

To a solution of 2-phenyl-maduraphthalazin-1-one **21** (281 mg, 0.5 mmol) in 5 ml of 1 M NaOH at 0~5°C was added 0.5 ml of methyl chloroformate. The mixture was stirred at room temperature for 30 minutes and than extracted with chloroform. The organic phase was washed with water, dried and concentrated. The product was precipitated with benzene to give light-red crystals of **25**.

^{1}H NMR (DMSO-d_6) δ 2.25 (3H, s, 11-CH_3), 2.80, 3.05 (2×2H, s, 6-, 7-CH_2), 3.9 and 4.0 (2×3H, s, 8-OCH_3, 12-$OCOOCH_3$), 7.48~7.75 (6H, m, 6×CH), 7.15 (1H, s, 1-CH), 8.3 (1H, s, 4-CH), 13.25, 13.60, 14.21 (3×1H, s, 10-, 15-, 16-OH).

^{13}C NMR ($CDCl_3$) δ 9.23 (11-CH_3), 8.28, 7.61 (6-, 7-CH_2), 55.96, 61.58 (8-OCH_3 and 12-$OCOOCH_3$), 112.25 (CH), 112.48, 113.86 (CH), 114.28, 120.56, 125.71, 127.21, 128.33, 128.92, 129.91, 130.09, 131.12, 139.71 (4-CH), 140.55, 146.60, 149.02, 151.15, 152.98, 154.41, 158.28, 158.55, 162.80, 163.15, 186.99, 187.22 (9-, 14-C).

2-Phenyl-10,12-dimethyl-maduraphthalazine-1-one **27** and 2-Phenyl-16(or 15)-trimethyl-maduraphthalazine-1-one **26**

A solution of 2-phenyl-maduraphthalazin-1-one (1.1 g, 2 mmol) **21** in DMF was converted with of sodium hydride (0.5 g. 80% in white oil) in it's sodium salt under vigorous stirring. After the gas evolution had finished iodomethane (0.5 ml, 8 mmol) was added dropwise. The color changed from blue-violet to dark red and the mixture was allowed to stay at room temperature over night. The mixture was crushed on ice and the precipitant was separated by centrifugation, washed with water several times and dried. The yellow brownish solid was purified by column chromatography. Three substances were separated: dihydroxy compound 86 mg (0.15 mmol, 8%), monohydroxyphenyl-maduraphthalazine 162 mg (0.27 mmol, 14%) and penta-methoxyphenylmaduraphthalazine 105 mg (0.17 mmol, 9%), the latter one is characterized in the next prescription.

Dihydroxy Compound **27**

^{1}H NMR ($CDCl_3$) δ 2.26 (3H, s, 11-CH_3), 2.3~3.6 (4H, m, 6-, 7-CH_2), 3.919, 3.923, 4.00 (3×3H, s, 8-, 10-, 12-OCH_3), 7.14 (1H, s, CH), 7.4~7.7 (6H, m, 6×CH), 8.26 (1H, s, CH), 13.15, 13.88 (2×1H, s, 15-, 16-OH).

^{13}C NMR ($CDCl_3$) δ 9.282 (11-CH_3), 22.881, 30.342 (6-, 7-CH_2), 56.095, 61.517, 62.022 (8-, 10-, 12-OCH_3), 103.481 (CH), 112.432, 113.816 (CH), 114.298, 120.991, 121.418, 125.428, 125.743 (CH), 127.401, 128.246 (CH), 128.888 (CH), 129.185, 129.601, 133.344, 139.754 (CH), 140.683, 146.414, 149.042, 149.687, 157.073, 158.319, 159.784, 162.181, 163.226, 181.320, 188.411.

Monohydroxy Compound **26**

^{1}H NMR ($CDCl_3$) δ 2.27 (3H, s, 11-CH_3), 2.40 (1H, t, J=8 Hz), 2.71 (1H, t, J=8 Hz), 3.04 (1H, d, J=8 Hz), 3.53 (1H, d, J=8 Hz, 6-, 7-CH_2), 3.72, 3.93, 3.40, 4.02 (4×3H, s, 8-, 10-, 12-, 16 (or 15)-OCH_3), 7.3~7.7 (6H, m, 6×CH), 8.16 (1H, s, CH), 13.80 (1H, s, 15 (or 16)-OH).

^{13}C NMR ($CDCl_3$) δ 9.28 (11-CH_3), 22.95, 30.12 (6-, 7-CH_2), 56.15, 61.52, 62.21, 62.97 (8-, 10-, 12-, 16 (or 15)-OCH_3), 103.61, 118.98, 125.94, 127.52, 128.61, 129.19, 131.74, 133.33, 137.45, 142.10, 146.29, 147.63, 149.54, 157.15, 159.75, 162.26, 181.34, 188.15 (9-, 14-CH).

2-Phenyl-,10,12,15,16-tetramethyl-maduraphthalazin-1-one **28**

To a solution of 2-phenyl-maduraphthalazin-1-one **21** (281 mg, 0.5 mmol) in dimethylformamide (16 ml, dried with molecular sieves 0.4 nm) at 0°C sodium hydride (300 mg) was added . The blue violet mixture was stirred for 5 minutes, iodomethane (3 ml) was added and stirring was proceeded for 1 hour at 50°C. The colour changed to light brown. After standing over night water was added to destroy the excess hydride and the solvent was evaporated. To the residue water and 2 M HCl (0.1 ml) were added. The crude product was purified by preparative thin layer chromatography (Merck plates, 2 mm (eluent: $CHCl_3$-CH_3OH, 20 : 1 v/v)). The zone with Rf=0.76 was isolated and extracted with tetrahydrofurane and concentrated. The product was precipitated with water to give yellow substance of **28** (280 mg, 90%), mp 251~257°C.

^{1}H NMR ($CDCl_3$) δ 1.3 (3H, s, 11-CH_3), 2.3~2.8 (2×2H, s, 6-, 7-CH_2), 3.68, 3.71, 3.93, 3.97, 3.99 (5×3H, s, 8-, 10-, 12-, 15-, 16-OCH_3), 7.3~7.5 (5H, m, 5-CH), 7.58, 7.50 (2×1H, s, 5-CH, 13-CH), 8.34 (1H, s, 4-CH).

^{13}C NMR ($CDCl_3$) δ 9.16 (11-CH_3), 23.29, 30.49 (6-, 7-CH_2), 56.14, 61.71, 62.60, 62.69, 63.03 (8-, 10-, 12-, 15-, 16-OCH_3), 103.69, 118.91 (5-, 13-CH), 126.04, 127.45, 127.64, 128.18, 128.74, 131.86, 132.27, 135.42, 137.51 (4-CH), 142.18, 143.29, 147.72, 151.90, 159.02, 162.37, 182.36, 182.68 (9-, 14-C).

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