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TWO CARBOXY- AND TWO HYDROXYMETHYL-SUBSTITUTED ARISTOLOLACTAMS FROM *ARISTOLOCHIA ARGENTINA*

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Key Word Index—*Aristolochia argentina*; Aristolochiaceae; aristololactam alkaloids; structural determination.

Abstract—Four new aristololactams have been isolated from *Aristolochia argentina*. The evidence indicates them to be 10-amino-3-hydroxymethyl-2,4-dimethoxyphenanthrene-1-carboxylic acid lactam, 10-amino-3-hydroxymethyl-2,4,6-trimethoxyphenanthrene-1-carboxylic acid lactam, 10-amino-2-hydroxy-4-methoxyphenanthrene-1,3-dicarboxylic acid lactam and 10-amino-2-hydroxy-4,6-dimethoxyphenanthrene-1,3-dicarboxylic acid lactam.

INTRODUCTION

In previous papers [1, 2] of this series, the isolation of 12 aristololactam alkaloids from *Aristolochia argentina* was reported. The present paper deals with four new aristololactams which contain hydroxymethyl and carboxyl groups as substituents.

RESULTS AND DISCUSSION

Aristololactam CII (**1**), $C_{18}H_{15}NO_4$ ($[M-1]^+$, m/z 308) and aristololactam CIII (**3**), $C_{19}H_{17}NO_5$ ($[M-1]^+$, m/z 338) were found in the neutral fraction of the ethanol extract of the rhizomes accompanying the non-phenolic aristololactams. Examination of the ^{1}H NMR data of aristololactam CII (Table 1) and those of reference compounds [2] revealed this compound to be a 2,3,4-trisubstituted aristololactam, two of the substituents being methoxy groups. The third substituent is a CH_2OH group as indicated by a two-proton singlet at *ca* δ5 in the ^{1}H NMR spectrum and a carbon signal at δ55.1 (DMSO-d_6) which splits to a triplet ($^1J_{CH}$ = 144 Hz) in the fully ^{1}H-coupled carbon spectrum. Accordingly, aristololactam CII affords a diacetate (**2**) by treatment with acetic anhydride–pyridine. Evidence for the relative location of the CH_2OH group and the two methoxy groups in aristololactam CII was mainly inferred from carbon shifts (Table 2). Since the CH_2OH substituent is expected to slightly protect *ortho*- and *meta*-carbons [3], the usual 3,4-dioxygenated pattern of aristololactams should be ruled out for aristololactam CII. The shifts of the oxyaromatic carbons in this compound are better accommodated in terms of a *meta*-relationship between the two oxygenated functions. Consistent with a 2,4-dimethoxy substitution pattern, C-4 resonates at δ155.0 (DMSO-d_6), i.e. *ca* $\Delta\delta$5 downfield as compared with C-4 of 3,4-dioxygenated lactams [2]. Further evidence comes from the methoxy carbon shifts. The OMe-4 group of aristololactam CII resonates at δ60.2 (DMSO-d_6), i.e. close to the δ59.4–60.0 range found for this group in several aristololactams. In contrast, the second methoxy group of aristololactam CII appears at δ61.6, which is quite distinct from the *ca* δ57 position expected for the OMe-3 signal, and is, therefore, assigned to C-2. Thus, aristololactam CII can be formulated as 10-amino-3-hydroxymethyl-2,4-dimethoxyphenanthrene-1-carboxylic acid lactam (**1**). The principal cleavages observed in the mass spectrum of aristololactam CII are depicted in Scheme 1. The mass spectrum typical

Table 1. ^{1}H chemical shifts of compounds **1**–**5** and **7**

Compound	Solvent	H-5	H-6	H-7	H-8	H-9	OMe-2	OMe-4	OMe-6	$HOCH_2$-3	NH
1	$CDCl_3$	9.08 *m*	7.56 *m*	7.56 *m*	7.75 *m*	7.20	3.99	4.14	—	5.25	9.42
1	DMSO-d_6	9.18 *m*	7.62 *m*	7.62 *m*	8.01 *m*	7.28	3.99	4.12	—	5.07	11.11
2*	DMSO-d_6	9.10 *m*	7.66 *m*	7.66 *m*	7.99 *m*	8.24	4.00	4.14	—	5.52	—
3	DMSO-d_6	8.59 *d*	—	7.24 *dd*	7.87 *d*	7.19	3.99	4.15	3.96	5.04	10.91
4	DMSO-d_6	9.02 *m*	7.61 *m*	7.61 *m*	7.91 *m*	7.27	—	4.10	—	—	†
5‡	$CDCl_3$	9.20 *m*	7.56 *m*	7.56 *m*	7.81 *m*	7.03	—	4.13	—	—	—
7§	$CDCl_3$	8.78 *d*	—	7.22 *dd*	7.75 *d*	7.02	—	4.17	3.98	—	—

δ-Values from TMS. *J* (Hz): 5,6 = 6,7 = 7,8 = 8.5; 5,7 = 2.5. Singlets not denoted.
*δ2.11 and 2.66 (3H, *s*, MeCO).
†δ12.5 broad signal (3H, CO_2H, OH, NH).
‡δ1.36 (3H, *t*) and 3.96 (2H, *c*) (NCH_2Me), 1.43 and 1.47 (3H, *t*), and 4.28 and 4.58 (2H, *c*) (OCH_2Me).
§δ1.36 (3H, *t*) and 3.97 (2H, *c*) (NCH_2Me), 1.42 and 1.46 (3H, *t*), and 4.28 and 4.59 (2H, *c*) (OCH_2Me).

Table 2. ^{13}C chemical shifts of aromatic oxy carbons, methoxyl carbons and hydroxymethyl carbons in compounds **1**, **4**, **5** and **7**

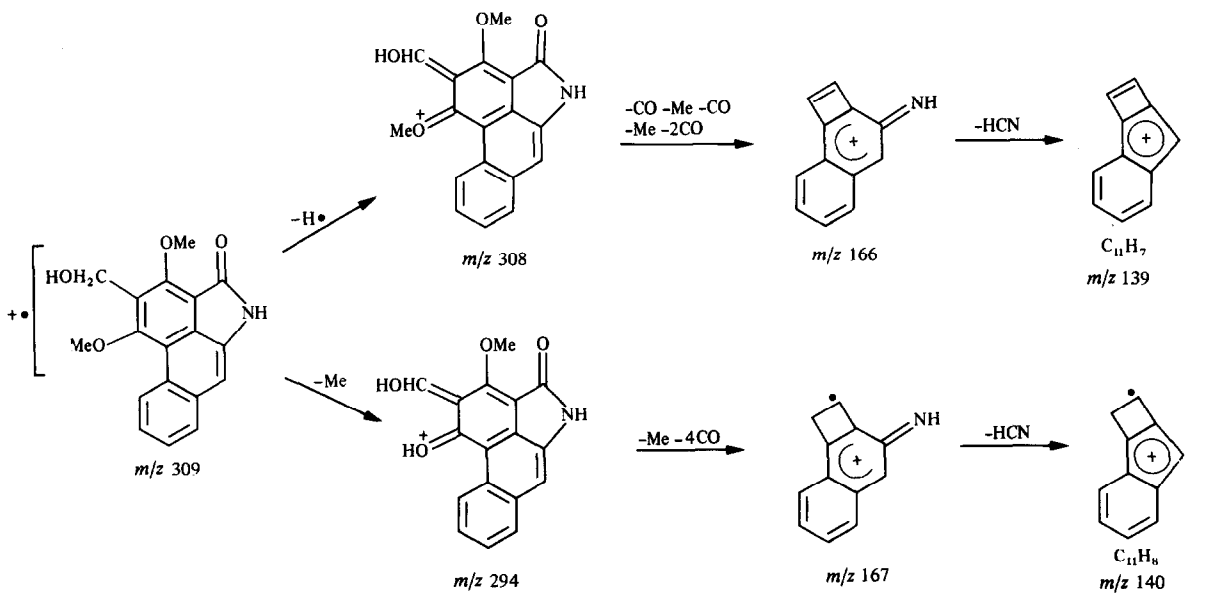
Compound	Solvent	C-2	C-4	C-6	OMe-2	OMe-4	OMe-6	$HOCH_2$-3
1	$CDCl_3$	150.7	155.1	—	61.1	59.8	—	57.5
1	DMSO-d_6	152.2	155.0	—	61.6	60.2	—	55.1
4	DMSO-d_6	*	155.3	—	—	60.0	—	—
5	$CDCl_3$	149.8	155.3	—	—	60.1	—	—
7	$CDCl_3$	149.9	155.1	157.7	—	60.3	55.4	—

δ-Values from TMS.
*Signal obscured by spectral noise.

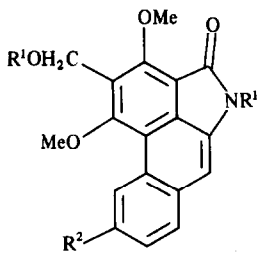
of aristololactams exhibits very strong $[M]^+$ peaks, usually the base peak, and the principal ions are associated with loss of methyl and carbonyl derived from initial cleavages around the methoxy functions. However, like benzyl alcohol [4], aristololactam CII shows a preferential loss of a hydrogen atom to give the base peak at *m/z* 308 followed by elimination of a carboxy group. Expulsion of a hydrogen atom from the CH_2OH group is more pronounced in aristololactam CII than in benzyl alcohol, presumably because the resultant ion is stabilized by the two adjacent methoxy groups. Initial expulsion of methyl may represent an equally likely process. The degradation path $[M-2Me\cdot-4CO-HCN]^+$ can be formulated in which the fission of the CH_2OH group proceeds, like benzyl alcohol, with migration of hydrogen atoms on the aromatic ring system. However, loss of one or two hydrogen atoms also occurs since the fragment ions are usually accompanied by the corresponding -1 and -2 ones. In contrast to typical aristololactams, the $[M]^+$ peak of aristololactam CII is not recognizable, possibly because of preferred stabilization through loss of one hydrogen atom and also by a more facile methyl elimination. Cleavage of one methoxy group with expulsion of a methyl radical may be favoured by a hydride transfer from the adjacent CH_2OH group to the ether oxygen in a manner analogous to the behaviour of *o*-methoxybenzyl alcohol [5].

Aristololactam CIII (**3**) was obtained as a minor component. The presence of an extra methoxy group located at C-6 was clearly shown by ^1H chemical shift data and the partition pattern (Table 1). Its mass spectrum exhibits the qualitative features expected from the breakdown pattern of aristololactam CII. An additional loss of methyl and carbonyl groups takes place in aristololactam CIII, ion **8** being observed as the main final hydrocarbon. Spectral data coupled with co-occurrence with aristololactam CII support structure **3** for aristololactam CIII.

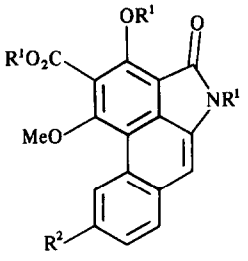
Two closely related fluorescent compounds were found in the fraction of non-phenolic aristolochic acids. Chemical and spectral evidence showed them to be phenolic carboxyaristololactams. The major compound, aristololactam DII (**4**), $C_{17}H_{11}NO_5$ ($[M]^+$ *m/z* 309), could be obtained as the free acid. Attempted methylation with ethereal diazomethane was unsuccessful, but prolonged treatment with diethyl sulphate–potassium carbonate led to a tri derivative (**5**), $C_{23}H_{23}NO_5$ ($[M]^+$ *m/z*



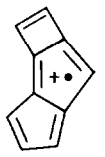
Scheme 1. Mass spectral fragmentation of aristololactam CII.



1 $R^1 = R^2 = H$
2 $R^1 = Ac$; $R^2 = H$
3 $R^1 = H$; $R^2 = OMe$

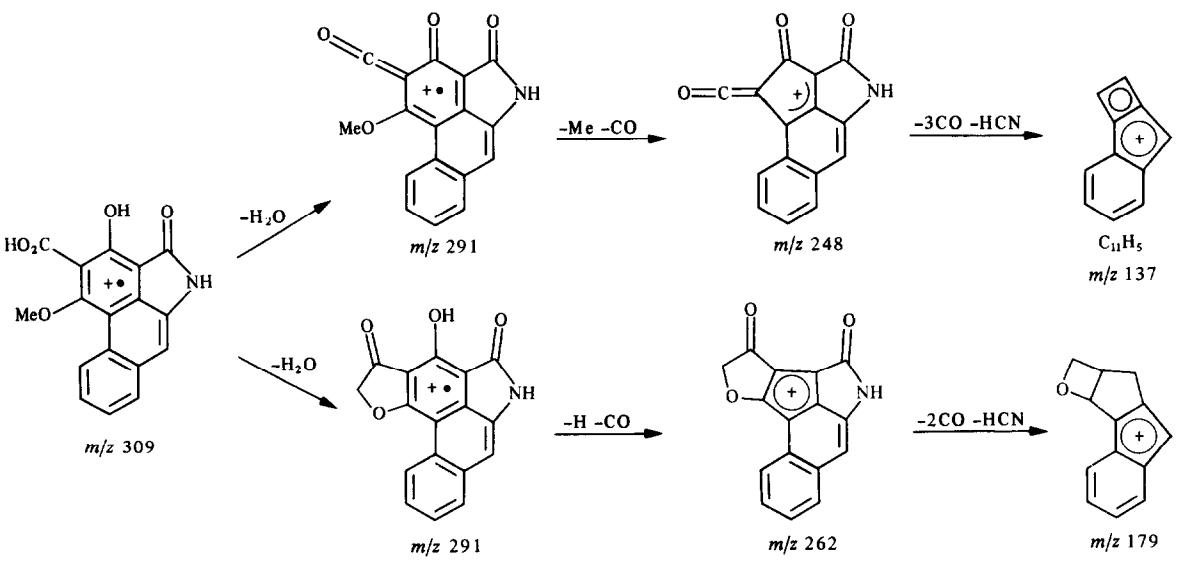


4 $R^1 = R^2 = H$
5 $R^1 = Et$; $R^2 = H$
6 $R^1 = H$; $R^2 = OMe$
7 $R^1 = Et$; $R^2 = OMe$



$C_{10}H_6$
m/z 126
8

393). ^1H NMR analysis (Table 1) showed that the carboxyl and hydroxyl groups and a methoxy group are located at positions 2–4 of aristololactam DII. The UV spectrum of **5** is closely comparable with that of aristololactam CII. However, aristololactam DII shows a marked displacement of bands toward longer wavelengths. This bathochromic effect may be explained in terms of contribution of *ortho*-quinoid structures due to intramolecular chelation between the OH-2 and the lactam carbonyl, as already known in the case of benzochromone derivatives [6]. The carboxyl group is assigned *ortho* to the hydroxyl because the mass spectrum of aristololactam DII shows a strong dehydration peak at *m/z* 291. The 2-hydroxy-3-carboxy-4-methoxy substitution pattern in aristololactam DII is further supported by its mass spectral fragmentation and carbon shifts. Assignment of the oxyaromatic carbons in compounds **4** and **5**, as shown in Table 2, was inferred by the similarity of the shifts with those of aristololactam CII, since the carboxyl and CO_2Et groups essentially do not affect resonances of *ortho*- and *meta*-carbons [3]. The low-field position of C-4 is interpreted, as in **1**, in terms of deshielding because of a *meta*-arrangement of the oxygenated functions. The signal for the methoxy carbon of aristololactam DII consistently appears at δ60.0 which is in accord with δ59.4–60.0 found for OMe-4 resonances of known aristololactams. Structural information was also obtained from the fragmentation pattern of aristololactam DII (Scheme 2). This compound behaves like typical *o*-hydroxybenzoic acids [7, 8] in that it shows a preferential loss of water to give the most abundant fragment species at *m/z* 291. The primary cleavage of the methoxy group with loss of methyl observed in aristololactams, is noted only to a very small extent in aristololactam DII. However, the fragmentation pattern below *m/z* 291 is very similar to that of the typical aristololactams, the main degradation routes being $[M - H_2O - Me - 4CO - HCN]^+$ and $[M - H_2O - 3CO - Me - CO - HCN]^+$. An alternative mode of breakdown in aristololactam DII occurs through dehydration between the carboxyl and methoxy groups with cyclization to a new ring, a process also observed in *o*-methoxybenzoic acid [8]. The resulting fragment (*m/z* 291) then suffers the successive losses of hydrogen, three carbonyls and hydrogen cyanide, as indicated by fragment ions at *m/z* 290, 262, 234, 206 and 179, respectively. These findings are compatible with a carboxyl group *ortho*-located to both hydroxyl and methoxy groups, and support structure **4** for this compound. The mass spectrum of **5** shows the $[M]^+$ at *m/z* 393 (base peak) and important fragments derived from initial cleavages around the ethoxy and CO_2Et groups. The most favoured



Scheme 2. Mass spectral fragmentation of aristolactam DII.

cleavage involves loss of ethene from the ethoxy group. The resultant ion, like methyl salicylate [8], then loses ethanol and a carbonyl group. As indicated by abundant ions at m/z 319, 291 and 276, the main degradation path of **5** proceeds through successive losses of ethene, ethanol, carbonyl, methyl and carbonyl. Important peaks at m/z 320 and 292 show that loss of an ethoxy group followed by a carbonyl or directly of the whole CO_2Et group from the $[M]^+$ or $[M-C_2H_4]^+$, processes already described for methyl salicylate [8], also occur. An initial MacLafferty elimination of ethene from the CO_2Et group can also take place giving an acid which, in turn, eliminates the elements of water, as in **4**. Peaks at m/z 347, 332 and 304 suggest the following decomposition sequences: $[M-C_2H_4-H_2O-C_2H_4-CO]^+$ and $[M-C_2H_4-H_2O-Me-CO]^+$. The fragment ion at m/z 349 may be attributed to elimination of acetaldehyde or an equivalent fragment from the CO_2Et group of **5** which then loses ethene and carboxyl. Initial loss of an ethyl radical from the ethoxy function originates alternative decomposition paths: $[M-Et-C_2H_4-H_2O-CO]^+$ and $[M-Et-C_2H_4-CH_2O]^+$. On the basis of the above evidence, aristololactam DII is formulated as 10-amino-2-hydroxy-4-methoxyphenanthrene-1,3-dicarboxylic acid lactam (**4**).

Aristololactam DIII (**6**) is present in very small amounts and could only be characterized as the *N,O*-diethyl ethyl ester (**7**), $C_{24}H_{25}NO_6$ ($[M]^+$ m/z 423). Its fragmentation behaviour, which follows that of **5**, and its NMR characteristics (Tables 1 and 2) indicated that aristololactam DIII is the 6-methoxy analogue of aristololactam DII.

Aristololactams are supposed to originate in the plant by oxidation of aporphines [2]. The presence of a carboxyl group in aristololactams DII and DIII suggest that they might be biogenetically derived from 3-carboxyphenylalanine or 3-carboxy-4-hydroxyphenylalanine. Both non-protein amino acids are known to occur in some members of the families Iridaceae, Resedaceae and Leguminosae [9, 10] and are produced via the modified shikimate pathway [9]. On the other hand, aristololactams CII and CIII might either arise from aristololactams DII and DIII by reduction and methylation reactions or directly originate from the amino acids 3-hydroxymethylphenylalanine and 3-hydroxymethyl-4-hydroxyphenylalanine. Indeed, these amino acids have been found together with the corresponding 3-carboxy-substituted amino acids and it appears likely that, after the formation of 3-carboxy compounds by the modified shikimate pathway, reduction reactions further convert carboxyl to methoxy [9]. Thus, the aristololactams here described may represent a case of incorporation of non-protein amino acids into alkaloids. It should be noted that alkaloids, such as the aporphines thalphenine and bisnorthalphenine, and the phenanthrenes thaliglucine, thaliglucinone, thalixine and thalflavidine, isolated from *Thalictrum* sp. (Ranunculaceae) [11], may also be formulated as derived from 3-carboxy- and 3-hydroxymethylphenylalanine.

EXPERIMENTAL

Mps are uncorr. UV spectra were recorded in 95% EtOH and IR spectra in KBr discs. ^1H NMR spectra were measured at 60 or 80 MHz and ^{13}C NMR spectra at 20 MHz. MS were recorded at 70 eV. TLC was carried out with the following systems: (i) silica gel, C_6H_6–MeCOEt (17:3), two developments (aristololactam, R_f: CII 0.45; CIII 0.37); (ii) Al_2O_3, $CHCl_3$–EtOH (100:1), two developments (aristololactam, R_f: CII, 0.28; CIII, 0.25); (iii) Woelm Mg silicate, C_6H_6–MeCOEt (3:1) (aristololactam, R_f: CII, 0.51; CIII, 0.32); (iv) silica gel, $CHCl_3$–MeOH (97:3) (aristololactam CII diacetate, R_f 0.87); (v) silica gel, $CHCl_3$–EtOH (7:3) (aristololactam DII, R_f 0.34; aristolochic acid I, R_f 0.66); (vi) silica gel, $CHCl_3$–MeOH–HCO_2H (94:5:1) (aristololactam DII, R_f 0.48; aristolochic acid I, R_f 0.48); (vii) cellulose (Merck aluminium-backed sheets), $CHCl_3$–MeOH–25% NH_3 (20:7:1.5) (aristololactam DII, R_f 0.30; aristolochic acid, I, R_f 0.80); (viii) cellulose, *n*-BuOH–*n*-PrOH–H_2O–25% NH_3 (100:60:50:1) (aristololactam, R_f: DII, 0.44; DIII, 0.34; aristolochic acid I, R_f 0.53); (ix) silica gel, C_6H_6–Me_2CO–HCO_2H (90:7:1) (aristololactam, R_f: DII, 0.71; DIII, 0.64; aristolochic acid I, R_f 0.64); (x) silica gel, C_6H_6–MeCOEt (19:1), two developments (compound, R_f: **5**, 0.48; **7**, 0.42); (xi) silica gel, $CHCl_3$ (compound, R_f: **5**, 0.49; **7**, 0.45); (xii) Al_2O_3, C_6H_6, two developments (compound, R_f: **5**, 0.50; **7**, 0.20). Detection: yellow spots or bands; fluorescence on silica gel at 360 nm: aristololactam CII, light blue; CIII, yellow-green; DII, green-orange; DIII, orange-red; **5**, yellow-orange; **7**, orange-red.

Extraction and isolation of aristololactams. Ground rhizomes of *Aristolochia argentina* (24 kg, dry wt) were extracted with boiling petrol and EtOH. The EtOH extracts were evaporated to dryness (3.9 kg), suspended in a mixture of H_2O and Et_2O and, under agitation, the pH adjusted to 9.7 with conc. NH_3. The Et_2O phase was separated and the remaining aq. phase as well as insoluble material were further extracted with Et_2O. The Et_2O extracts were combined, extracted with H_2O buffered to pH 1.5 and evaporated to dryness. The oily residue (432 g), containing the neutral compounds, was submitted to repeated CC on silica gel and Al_2O_3 (C_6H_6–$CHCl_3$–MeOH) to give several fractions. The crude fraction of non-phenolic aristololactams (10.3 g) was further purified by extensive prep. TLC using system ii which, at the same time, allowed the separation of aristololactams CII and CIII from the accompanying lactams. Elution of the R_f 0.25–0.28 band afforded a crude mixture (2 g) of aristololactams CII and CIII. Their separation was achieved by repeated prep. TLC in system i yielding 128.5 mg aristololactam CII and 10.5 mg aristololactam CIII.

A fraction of non-phenolic aristolochic acids (15.1 g), corresponding to 13.3 kg rhizomes (dry wt), was obtained by counter-current distribution from the whole crude mixture of acids. A portion of the non-phenolic aristolochic acids was removed by crystallization (EtOH). The mother liquor (9.6 g), containing aristololactams DII and DIII (systems v–ix), was chromatographed on cellulose columns ($CHCl_3$–MeOH–25% NH_3, 100:9:1.5, 100:21:4.5, 100:31:7.5, etc.) in order to separate the remaining aristolochic acids. The fractions containing the aristololactams were further purified by CC (silica gel, $CHCl_3$–MeOH–HOAc, 95:4:1, 93:6:1, 91:8:1, etc.) and prep. TLC (system ix) to give 119.3 mg aristololactam DII.

2-Hydroxymethyl-1,3-dimethoxydibenz(cd,f)indol-4(5H)-one, aristololactam CII (**1**). Pale yellow needles (C_6H_6), mp 190°; UV λ_{max} nm (log ε): 230 sh (4.46), 233 (4.48), 258 sh (4.46), 266.5 (4.49), 288 (4.42), 335 sh (3.74), 352 (3.72), 384 (3.81); IR ν_{max} cm^{-1}: 1701, 1664, 1453, 1381, 1319, 1164, 1039, 1013, 988; EIMS m/z (rel. int.): 309 (20.8), 308 $[M-1]^+$ (100), 295 (9.0), 294 (53.3), 280 (8.9), 279 (47.3), 264 (12.1), 263 (8.5), 252 (8.0), 251 (46.6), 195 (7.5), 167 (9.5), 166 (7.5), 164 (8.9), 140 (7.7), 139 (8.6), 111 (8.6), 100 (17.7), 70 (37.1), 43 (9.7).

Diacetate (**2**). A mixture of aristololactam CII (7 mg), Ac_2O (0.6 ml) and C_5H_5N (0.6 ml) was allowed to stand at room temp. for 5 days. Pale yellow needles (7 mg) separated on drop-wise addition of H_2O, mp 207–208°.

2-Hydroxymethyl-1,3,9-trimethoxydibenz(cd,f)indol-4(5H)-

one, aristololactam CIII (**3**). Yellow needles (C_6H_6), mp 230–235°; UV λ_{max} nm (log ε): 234 (4.59), 264 (4.51), 291 (4.45), 342 (3.90), 357 (3.84), 398 (3.88); IR ν_{max} cm^{-1}: 1659, 1626, 1394, 1375, 1354; EIMS *m/z* (rel. int.): 339 (19.0), 338 $[M-1]^+$ (100), 325 (8.8), 324 (34.8), 310 (22.9), 309 (8.4), 295 (8.0), 293 (10.1), 281 (19.1), 280 (7.4), 266 (7.7), 126 (7.7), 70 (14.8).

4,5-*Dihydro*-3-*hydroxy*-1-*methoxy*-4-*oxodibenz*(cd,f)*indol*-2-*carboxylic acid, aristololactam DII* (**4**). Yellow needles (DMSO/*n*-PrOH), mp 278–280°; UV λ_{max} nm (log ε): 239 (4.43), 274 sh (4.44), 285 (4.60), 296 (4.55), 414 (3.82); IR ν_{max} cm^{-1}: 1663, 1615, 1439, 1403, 1371, 1304, 1240; EIMS *m/z* (rel. int.): 310 (11.3), 309 $[M]^+$ (62.0), 292 (17.9), 291 (100), 290 (7.2), 264 (13.2), 263 (74.6), 262 (20.6), 249 (8.8), 248 (52.1), 236 (5.3), 235 (28.0), 234 (4.5), 220 (5.0), 207 (9.8), 192 (6.3), 179 (5.1), 165 (6.3), 164 (32.5), 145.5 (7.4).

3-*Ethoxy*-5-*ethyl*-4,5-*dihydro*-1-*methoxy*-4-*oxodibenz*(cd,f)-*indol*-2-*carboxylic acid ethyl ester* (**5**). Half the amount of crystals of aristololactam DII and its mother liquor were separately ethylated (Et_2SO_4, McCOEt, K_2CO_3, 80°, 2 days [12]). Both reaction products were subjected to prep. TLC (systems x and xii) yielding a total of 117.6 mg **5**, yellow plates (C_6H_6–petrol), mp 121–122°; UV λ_{max} nm (log ε): 235 (4.51), 257 (4.50), 264 (4.50), 290 (4.45), 342 sh (3.59), 384 (3.76); IR ν_{max} cm^{-1}: 1718, 1701, 1639, 1373, 1309, 1237, 1047; EIMS *m/z* (rel. int.): 395 (4.4), 394 (28.7), 393 $[M]^+$ (100), 365 (5.1), 364 (19.6), 349 (9.1), 320 (14.4), 319 (27.7), 318 (13.4), 306 (8.9), 304 (9.7), 292 (8.8), 291 (37.6), 290 (9.8), 277 (4.5), 276 (17.2), 263 (7.4), 248 (4.2).

3-*Ethoxy*-5-*ethyl*-4,5-*dihydro*-1,9-*dimethoxy*-4-*oxodibenz*-(cd,f)*indol*-2-*carboxylic acid ethyl ester* (**7**). From the mother liquor of crystallization of aristololactam DII, after ethylation and prep. TLC (systems x and xii), 13.4 mg **7** was obtained, yellow needles (C_6H_6–petrol), mp 131°; UV λ_{max} nm (log ε): 232 (4.52), 238 (4.53), 264 (4.50), 297 (4.42), 344 (3.76), 357 (3.69), 403 (3.82); IR ν_{max} cm^{-1}: 1750, 1691, 1650, 1625, 1385, 1260, 1220; EIMS *m/z* (rel. int.): 424 (18.5), 423 $[M]^+$ (72.0), 380 (10.3), 379 (39.7), 350 (12.4), 349 (11.1), 306 (11.2), 305 (11.2), 279 (40.0), 277 (14.0), 267 (19.4), 150 (10.2), 149 (100), 113 (43.9), 97 (10.3), 84 (11.6), 83 (15.6), 71 (50.0), 70 (15.4), 69 (15.2), 57 (53.1), 56 (11.3), 43 (16.9).

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