MASS SPECTROMETRY OF ARISTOLOCHIC ACIDS

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ABSTRACT.—The electron impact-induced fragmentation of aristolochic acids and some synthetic model compounds has been investigated using high resolution mass spectrometry. It was found that the only primary cleavage given by condensed aromatic systems having a carboxy and a nitro function in *peri* positions consists of the elimination of NO_2. The complexity which is usually observed in the upper mass range of the spectra of aristolochic acids is attributable to contaminations with closely related compounds. The potential of high resolution mass spectrometry for the analysis of mixtures of aristolochic acids from natural sources is demonstrated.

In the course of recent work on aristolochic acids **1–6** from *Aristolochia chilensis* (1) and *Battus archidamas* (2) we found that high resolution mass spectrometry is a very valuable tool both for the identification of the pure acids and for the analysis of mixtures. The investigations also revealed that the nitroacids are usually contaminated with minute amounts of their lactam counterparts **7–12** which—although not detectable by other spectrometric methods (ir, ^1H nmr) or chromatography—give rise to peaks of medium to strong intensity, thus leading to rather complex spectra. From accurate mass measurements on a number of natural products and from the study of some model compounds the fragmentation pathways of aristolochic acids have been derived. The findings led to a substantial modification of the tentative interpretation given by Rothschild, von Euw and Reichstein (3) which was based on low resolution data of slightly inhomogeneous compounds.

Figure 1 shows the mass spectrum of a chromatographically pure sample of

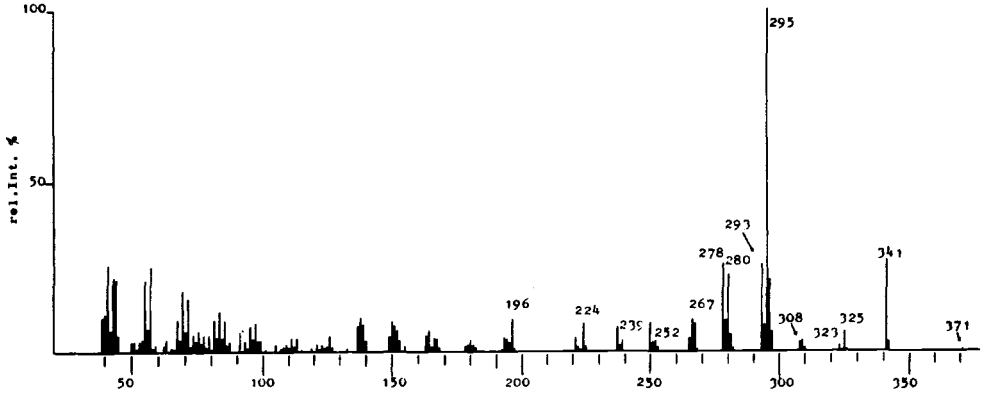
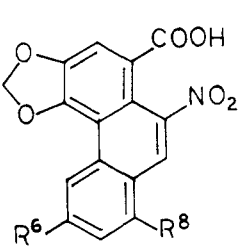
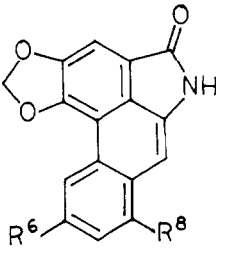


FIG. 1. Mass spectrum of chromatographically pure aristolochic acid I (**3**).

aristolochic acid I (**3**) taken half an hour after full insertion of the probe into the ion source at a temperature of 200°. The elementary composition of the molecular ion at *m/z* 341 was determined as $C_{17}H_{11}NO_7$. As can be concluded from the high resolution data of the other peaks, the only primary fragmentation of $M^{+\cdot}$ consists of the elimination of an NO_2 radical yielding the base peak at *m/z* 295 ($C_{17}H_{11}O_5^+$), which then fragments further by consecutive loss of small units

such as H, CH_3, CO, CHO, to the ions $C_{17}H_{10}O_5^{+\cdot}$ (part of 294), $C_{16}H_8O_5^{+\cdot}$ (280), $C_{16}H_{11}O_4^+$ (267), $C_{15}H_8O_4^{+\cdot}$ (252), $C_{15}H_{11}O_3^+$ (239), $C_{14}H_8O_3^{+\cdot}$ (224), $C_{13}H_8O_2^{+\cdot}$ (196). Other primary fragmentations that have been postulated (3) like the elimination of O, H_2O, NO, CH_2O, COOH, etc. do not occur, which means that the peaks at such mass distances have to be attributed to impurities, possibly homologous compounds. Accordingly, a small amount (ca 2%) of the more highly substituted aristolochic acid IV (**6**) with $M^{+\cdot}$ $C_{18}H_{13}NO_8$ (*m/z* 371) leads to the $(M-NO_2)^+$ fragment $C_{18}H_{13}O_6^+$ at *m/z* 325.





	R^6	R^8	
1	H	H	**7**
2	H	OH	**8**
3	H	OCH_3	**9**
4	OH	OH	**10**
5	OH	OCH_3	**11**
6	OCH_3	OCH_3	**12**

For *m/z* 293 an elementary composition $C_{17}H_{11}NO_4$ was found. A thermal or electron impact-induced loss of three oxygen atoms from $C_{17}H_{11}NO_7$ seemed very unlikely, especially since the relative intensity of *m/z* 293, which had been much larger just after introduction of the probe into the ion source, slowly decreased with time, and since no metastable transition for such a process could be detected. We concluded therefore that *m/z* 293 represents the molecular ion of the equally substituted lactam **9**, present in the sample in an unknown quantity. **9** can easily split off a CH_3 radical from the methoxy group to give $C_{16}H_8NO_4^+$ (278) with favourable stabilization of the positive charge by a quinoidal ion structure. Analogously, *m/z* 323 ($C_{18}H_{13}NO_5^{+\cdot}$) is the molecular ion of the lactam **12** yielding the $(M-CH_3)^+$ fragment ($C_{17}H_{10}NO_5^+$ (308).

This interpretation can successfully be used for the analysis of more complex mixtures. As an example, figure 2 shows the mass spectrum of an apparently homogeneous fraction which was obtained after paper chromatography in one solvent system of an acid extract of *A. chilensis*. The high resolution data

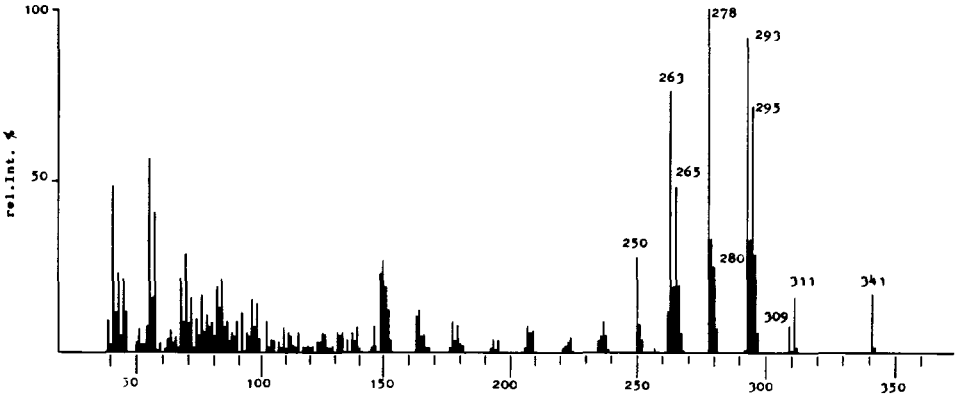


FIG. 2. Mass spectrum of impure aristolochic acid I (**3**).

revealed the presence of **3** ($M^{+\cdot}$, 341; $(M-NO_2)^+$, 295; $(M-NO_2-CH_3)^{+\cdot}$, 280), **1** ($M^{+\cdot}$, 311; $(M-NO_2)^{+\cdot}$, 265), the corresponding lactams **9** ($M^{+\cdot}$, 293; $(M-CH_3)^+$, 278; $(M-CH_3-CO)^+$, 250), **7** ($M^{+\cdot}$, 263) and a trace of **5** ($M^{+\cdot}$, 357 measured by peak-matching; $(M-NO_2)^+$, part of 311) and its lactam counterpart **11** ($M^{+\cdot}$, 309). After several crystallizations, **3** was obtained practically free of all contaminants except **9**.

In order to verify this interpretation the three model compounds **13–15** were synthesized (4) and their fragmentations studied. The 5-nitroacid **13**, with no interaction between the functional groups, gives a mass spectrum (figure 3) as

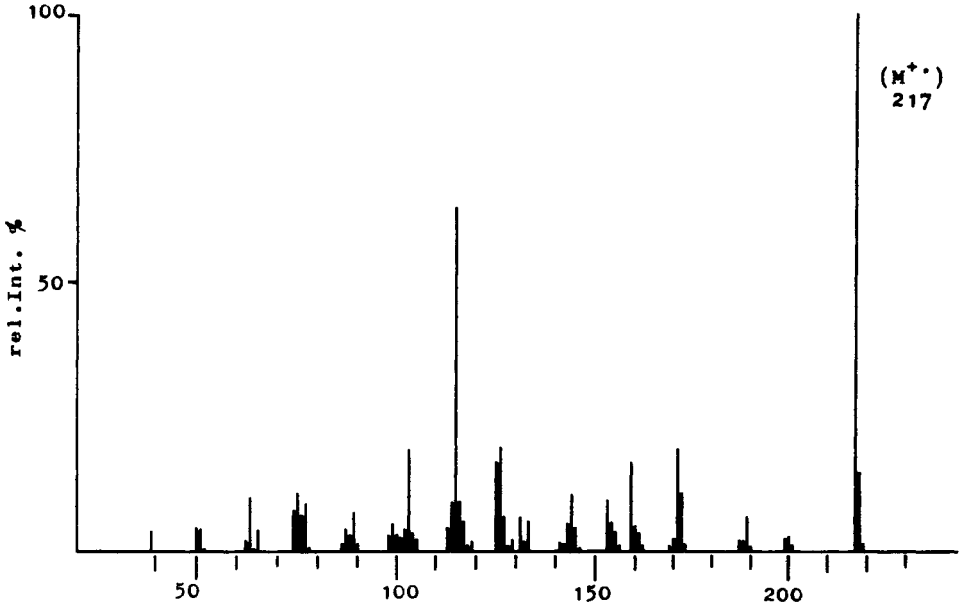


FIG. 3. Mass spectrum of 5-nitro-1-naphthoic acid (**13**).

can be expected from its structure, a very intense molecular ion ($C_{11}H_7NO_4^{+\cdot}$, 217), followed by a large number of weak primary fragments $(M-O)^{+\cdot}$, $(M-OH)^+$, $(M-H_2O)^{+\cdot}$, $(M-CO)^{+\cdot}$, $(M-CHO)^+$, $(M-NO)^{+\cdot}$, $(M-CO_2)^{+\cdot}$, $(M-NO_2)^+$, which decompose further by loss of small units.

In marked contrast, the 1,8-isomer **14** very easily splits off an NO_2 radical from its molecular ion, giving the base peak ($C_{11}H_7O_2^+$, *m/z* 171, the only primary fragment of importance (figure 4). Subsequently, a one- or two-step elimination of two molecules of CO leads to $C_{10}H_7O^+$ (143) and $C_9H_7^+$ (115), and loss of COOH to $C_{10}H_6^{+\cdot}$ (126). Exactly the same cleavage pattern was observed by M. Pailer et al. (5) with the methyl and ethyl esters of a series of aristolochic acids. It results from a neighbouring group interaction of the *peri* substituents which is, however, quite different from the *ortho* effect described for *o*-nitrobenzoic acid (6).

Figure 5 shows the spectrum of the lactam **15**. Most of the total ion current is concentrated in the molecular ion $C_{11}H_7NO^{+\cdot}$ (169). The main fragments are $C_{10}H_7N^{+\cdot}$ (141), $C_{10}H_6N^+$ (140), $C_9H_6^{+\cdot}$ (114), and $C_9H_5^+$ (113).

As stated before, all samples of aristolochic acids showed peaks in their mass spectra originating from aristolactams. Regardless of the purity of the samples, only lactams with the same substituents patterns as the aristolochic acids present could be identified, but none whose acid counterparts were lacking. The intensity of the mentioned peaks was often very strong, which was rather surprising since no such lactams were detected by ir, ^1H nmr or chromatography, and consequently must be present in only small amounts. Therefore, in a further experiment a sample consisting of 99% **14** and 1% **15** was fully introduced into the ion source

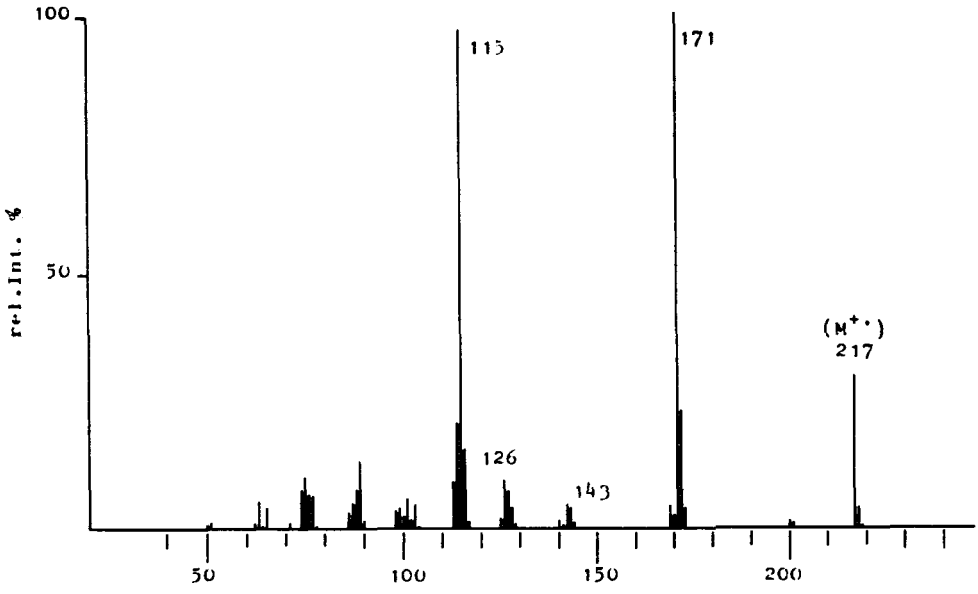


FIG. 4. Mass spectrum of 8-nitro-1-naphthoic acid (**14**).

and the spectra were recorded over a period of time. The following intensities for $M^{+\cdot}_{acid}$:$(M_{acid}-NO_2)^+$:$M^{+\cdot}_{lactam}$ were measured: after 0'30", 13:54:100; after 1'30", 26:100:48; after 4'30", 31:100:12, and after 8'30", 29:100:11. The data clearly demonstrate that minor contamination of the acid by its lactam will lead to a considerable $M^{+\cdot}_{lactam}$ peak—which can even be the base peak in the

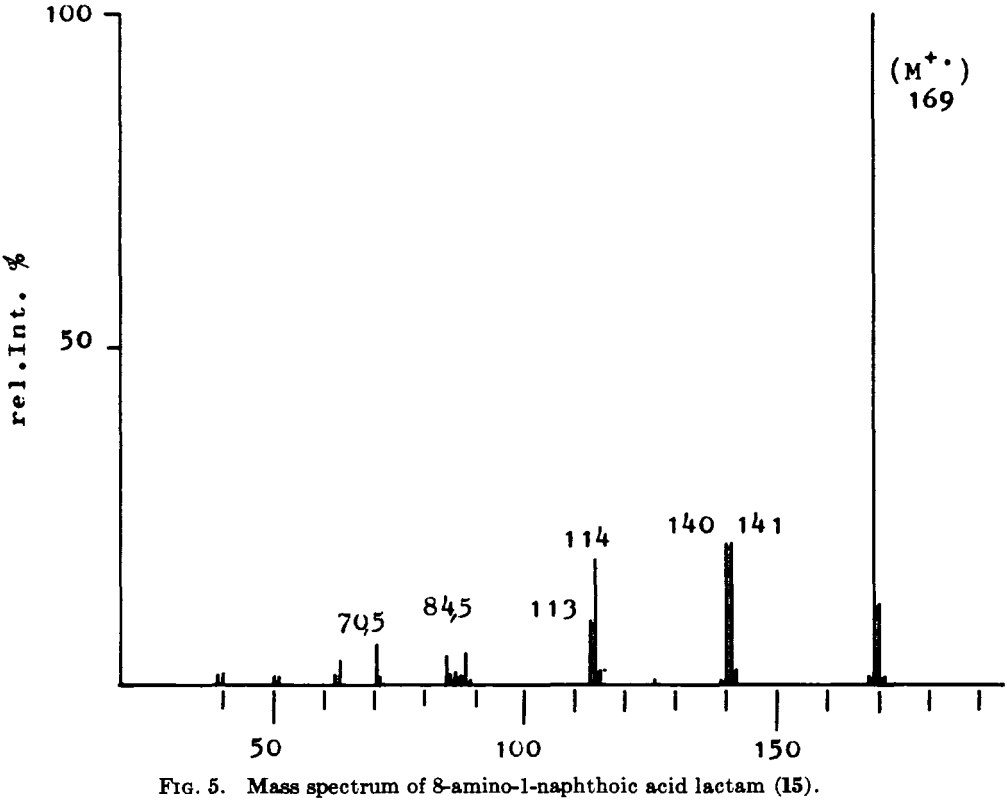
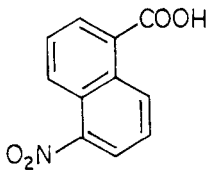


FIG. 5. Mass spectrum of 8-amino-1-naphthoic acid lactam (**15**).



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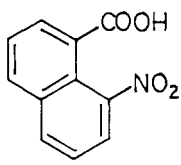

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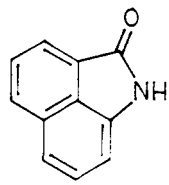

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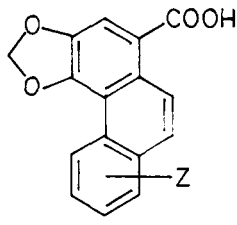

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mass spectrum—thus suggesting a far greater degree of impurity than is actually the case. Nitroacids of this structural type are very easily reduced to the lactams. In the case of **14**, the reduction is instantly achieved at room temperature by Fe^{2+} ions in aqueous solution (4). We conclude therefore that traces of aristolactams are generated in a similar way from the aristolochic acids during and even after the isolation and purification processes.

One other problem regarding the interpretation of the mass spectra of the natural products has to be mentioned. As can be seen in figure 2, the peaks at *m/z* 296 and 266—and to a much lesser extent also at 297 and 267—are too large to be solely the ^{13}C isotopic peaks of the respective $(M-NO_2)^+$ fragments. In the published spectra of **2** (two isomers) (3) and in the spectra of the phenolic aristolochic acids **2** and **5** measured by us, analogous peaks (45 and 44 mass units below $M^{+\cdot}_{nitroacid}$, respectively) are to be seen. By accurate mass measurements we could prove that in all cases the extra intensity of the formal "(M–45)" ions was *not* caused by the loss of COOH from the nitro acids, but stemmed from nitrogen-free ions of the general formula **16**. At an ionization energy of 12 eV a relative increase, and after heating the probes in the ion source for some time a decrease in the intensity of these ions was observed, which indicates that they represent yet another type of contaminant. The process $M^{+\cdot}+H\cdot-NO_2\cdot$, involving an ion-molecule reaction, cannot entirely be excluded however. As to the much less important "(M–44)" peaks, it was found that they originate from decarboxylated species related to the aristolochic acids. Whether they are genuine impurities or are produced by thermal loss of CO_2 (6) was not studied.

EXPERIMENTAL

Mass spectra were obtained with an MS-50 mass spectrometer and on-line DS-50 data system (Kratos) using direct sample insertion into the ion source (temperature between 150 and 250°; ionization energy 70 eV). For the isolation of the aristolochic acid samples, see reference (1). **13–15** were prepared by published procedures (4).

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