Spectroscopic Properties of Some Derivatives of Polycyclic Aromatic Hydrocarbons

ALFIO CATALFO, MARIA ELISABETTA SERRENTINO, VITO LIBRANDO, GIANCARLO PERRINI, and GUIDO DE GUIDI*

Dipartimento di Scienze Chimiche, Università di Catania, Viale Andrea Doria 6, 95125, Catania, Italy

The aim of this paper is to provide a general picture of the spectral characteristics of some polycyclic aromatic hydrocarbon (PAH) derivatives. A great deal of data concerning PAHs has been reported in the literature, but there is lack of comprehensiveness about important parameters in the same experimental conditions for their nitro (NO_2) and amino (NH_2) derivatives such as absorption and emission characteristics. Thus, important parameters such as the molar extinction coefficient, absorption maxima, fluorescence maxima, and fluorescence quantum yield are reported here. The efficiencies of the reduction of NO_2-PAHs to their corresponding amino compounds were also verified by means of high-performance liquid chromatography (HPLC). This class of derivatives represents one of the most toxic groups of carcinogenic substances and therefore the data reported here should be useful for toxicological research.

Index Headings: **Polycyclic aromatic hydrocarbons; PAHs; NH_2-PAHs; Amino-polycyclic aromatic hydrocarbons; NO_2-PAHs; Nitro-polycyclic aromatic hydrocarbons; Absorption spectra; Emission spectra; Fluorescence; High-performance liquid chromatography; HPLC-FL; HPLC-MS.**

INTRODUCTION

Amino-polycyclic aromatic hydrocarbons (NH_2-PAHs) are one of the most toxic groups of carcinogenic substances.[1–8] Their structure is related to the polycyclic aromatic hydrocarbons (PAHs) and their toxic parent nitro derivatives (NO_2-PAHs), well known as environmental pollutants (Fig. 1). PAHs and their derivatives consist of many compounds produced during the incomplete combustion of organic matter. An alternative pathway through nitrosation of PAH to a nitro derivative seems to happen through contribution of free radicals in atmosphere produced via several paths, such as photochemical reactions; also, NH_2-PAHs are photochemically produced to a lesser extent.[9] The subsequent reactivity of nitroaryls depends on the electronegativity strength of the substituents.[9,10] Differently from PAHs, the presence of the nitro group greatly enhances the toxic spectrum of activity.[1]

Once absorbed, the NO_2-PAHs are reductively bio-transformed to NH_2-PAH followed by further conjugation and/or excretion in urine. Examples of biomarkers for exposure to NO_2-PAHs include 3-aminobenzanthrone and 1-aminopyrene, which are good indicators for diesel exhaust exposure.[1,11,12] This class of polar compounds (amino and nitro derivatives) exists in aqueous medium in two different forms depending on the pH of the solution; the more toxic aminos are more soluble in aqueous medium with respect to the nitro compound; at physiological pH they are mainly present in the ionic form.

The NO_2-PAHs are mainly formed in the atmosphere by radicals,[2] while their reduction to NH_2-PAHs often involves organisms.[3] Amino derivatives are not tolerated in cells and exhibit highly adverse effects by radical and other toxic product generation.[3]

Moreover, most NO_2-PAHs and NH_2-PAHs show absorption maxima in the UVA range; thus, they belong to a class of substances that are able to induce phototoxicity.[1] In addition to their role as atmospherically relevant photochemical radical sensitizers, they show photosensitizing properties toward cells.[2,4] Some of them can act as mutagenic and carcinogenic agents,[4] in which other secondary processes could take place. Not less important in the overall chemical picture are the oxidation of amino substituents, generation of singlet oxygen, and production of superoxide. Analysis of NO_2-PAHs and NH_2-PAHs in diesel exhaust, aerosol, and air particulate matter are often carried out by pre-fractionation followed by high-performance liquid chromatography (HPLC) or gas chromatography (GC) separation, where the possible detection methods include mass spectroscopy (MS), electrochemical detection (EC), fluorescent detection (FL), and ultraviolet–visible detection (UV-Vis).[4] Reverse-phase HPLC separation of NO_2-PAHs and NH_2-PAHs converted from their parent compounds by reduction is commonly used.[9,13] Thus, we have characterized some representative nitrated and amino derivatives of polycyclic aromatic hydrocarbons via absorbance, emission, and electrospray ionization mass spectroscopy (ESI-MS), coupled with electrochemical methods. These are simple and quite highly sensitive techniques.

Detailed data concerning absorbance and emission characteristics of amino derivatives are incomplete in the literature; some of these are difficult to find and many are lacking. In particular, the extinction coefficients, as well as emission behavior, coupled with ESI-MS identification, represent an important parameter for an unambiguous qualitative determination of these hazardous compounds in cells and tissues of organisms. Thus, the present work meets the increasing need for more detailed and comprehensive spectroscopic data about PAH derivatives such as NH_2-PAH.

EXPERIMENTAL

Chemicals. The nitro derivatives 1-NO_2P (1-nitropyrene), 2-NO_2F (2-nitrofluorene), 3-NO_2PAN (3-nitrophenanthrene), 9-NO_2PAN (9-nitrophenanthrene), 3-NO_2BPH (3-nitrobiphenyl), 7-NO_2BaAN (7-nitrobenzo(a)anthracene), 6-NO_2BaP (6-nitrobenzo(a)pyrene), 6-NO_2CHR (6-nitrochrysene), 9-NO_2AN (9-nitroanthracene), and 3-NO_2FA (3-nitrofluoranthene) were obtained from Accustandard (New Haven, CT). The PAHs P (pyrene), F (fluorene), PAN (phenanthrene), BPH (biphenyl), BaAN (benzo(a)anthracene), BaP (benzo(a)pyrene), CHR (chrysene), AN (anthracene), and FA (fluoranthene), as well as the amino derivatives 1-NH_2-P and 2-NH_2-F, were obtained from Sigma (Milano). Methanol, acetonitrile, and water were HPLC grade. All other chemicals were reagent grade. Stock

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* Author to whom correspondence should be addressed: gdeguidi@unict.it.

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solutions and working standards were prepared in methanol. All reagents, stock solutions, and working standards were stored refrigerated in the dark.

Analytical Instrumentation.. Ultraviolet-visible absorption spectra were recorded on a single-beam spectrophotometer Beckman DU 650, whereas emission spectra were obtained by means of a Spex Fluorolog-3, Horiba Jobin-Yvon spectrofluorimeter. Chemical reduction efficiencies were followed by HPLC (HP 1100) equipped with an on-line diode array detector (DAD), electrochemical (EC) detector (ESA 5100A), fluorescence (FL) detector (Kontron SFM), and mass spectrometer (MS) detector equipped with an API-ES interface (Agilent VL).

Chemical Reduction. The chemical reduction procedure is based on a method by Campbell and Lee with some modification and was carried out in the dark.[11,13] NO_2-PAH (about 10 μg) was dissolved in 1.6 mL of MeOH and 0.4 mL of copper(II)sulfate 0.04%; then, 20 mg KBH_4 was added to the reaction mixture and the reaction was followed by emission spectroscopy. Incubation for three hours at room temperature in the dark leads to almost complete reduction. Then, absorption, emission and HPLC/EC/FL/MS measurements were carried out immediately.

High-Performance Liquid Chromatography (Chemical Reduction Efficiencies and Product Identification). Twenty microliters (20 μL) of the reduced and non-reduced samples were injected into a reverse-phase Hypersil ODS C18 (120 Å, 5 μm, 4.6 × 100). Elution was carried out using 0.1 M sodium chloroacetate (pH = 3) with 50% ACN (v/v) at a rate of 1 mL/min. The parameters of the fluorescence detector were set as shown in Table I. The parameters of the electrochemical detector were set as follows: conditioning cell −1 V, detector I −0.5 V, detector II −0.6 V, response time 0.1 s, and gain 1 × 10. The mass spectrometer was set as follows: positive ion mode, SCAN (100–400 a.m.u.) or SIM (single ion monitoring) at various a.m.u. for NO_2-PAH and NH_2-PAH (see Table I). Nebulizer and curtain nitrogen gas 60 psig and 13 L/min respectively, drying gas at 350 °C, capillary voltage 3.5 kV, and fragmentor 100 V. Results are reported as molar concentration, after the correlation of the integrated peaks, of the opportune trace, with the respective standard curve.

RESULTS AND DISCUSSION

Much of the data concerning the spectroscopic characteristics of NH_2- and NO_2-PAH derivatives is incomplete in the literature. PAHs and their derivatives are a class of environmental contaminants that have been of interest for a long time in organic, theoretical, and physical chemistry, as well as in environmental science, toxicology, cancer research, and energy sciences. Concerning environmental science and cancer research, the majority of the research has focused on occurrence, environmental fate, degradation/remediation, chemical transformation, genotoxicity, metabolism and metabolic activation, DNA adduct formation, mutagenesis, and carcinogenesis. The toxic properties of NH_2-PAHs have been attributed to the formation of DNA cross-links, such as DNA adducts, which are responsible for the modification of genome.[11] The formation of covalent NO_2-PAH DNA adducts and NO_2-PAH mediated oxidative lesions are two possible mechanisms for the initiation of NO_2-PAH carcinogenesis.[14] Indeed, DNA modifications represent one of the major classes of cell damage induced by PAHs.[15] Guanine moieties have been shown to be the most susceptible DNA target with regard to oxidation induced by ROS (reactive oxygen species), which are considered responsible for mutagenic and carcinogenic events.[14] Several authors have reported the correlation between the effect of NO_2-PAHs metabolic reduction with the ability of

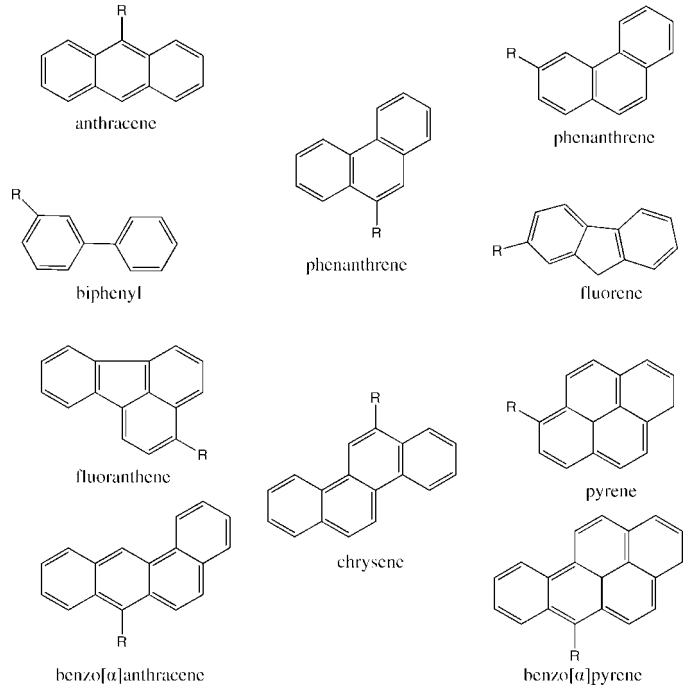

FIG. 1. Chemical structure of PAHs and their derivatives used in this study: R = H; (PAH); R = NO_2; (NO_2-PAH); R = NH_2; (NH_2-PAH).

TABLE I. Values of the a.m.u., reduction yields, half-wave potentials, and retention times.

PAH	RT (min)	a.m.u.	NO_2-PAH	a.m.u. MH^+	RT (min)	$E_{1/2}$ mV[a]	NH_2-PAH	Yield %	RT (min)	a.m.u. MH^+
P		202	1-NO_2P	248	21	−440	1-NH_2P	98	8.2	218
F^-	13.2	166	2-NO_2F^-	212	12.2	−620	2-NH_2F	100	5.8	182
PAN	15.2	178	9-NO_2PAN	224	2.8		9-NH_2PAN	99	5.1	194
PAN	15.2	178	3-NO_2PAN	224	5.5		3-NH_2PAN	98.5	5.5	194
BPH	11.3	154	3-NO_2BPH	200	2.1		3-NH_2BPH	99	4.0	170
BaAN		228	7-NO_2BaAN	274	1.8	−580	7-NH_2BaAN	94	1.0	244
BaP	73.9	252	6-NO_2BaP	298	61.5	−530	6-NH_2BaP	91	19.4	268
CHR	13.0	228	6-NO_2CHR	274	35.4	−430	6-NH_2CHR	96	11.1	244
AN	17.4	178	9-NO_2AN	224	13	−570	9-NH_2AN	91	6.3	194
FA		202	3-NO_2FA	248	21.8	−390	3-NH_2FA	100	7.4	218

[a] Ref. 19.

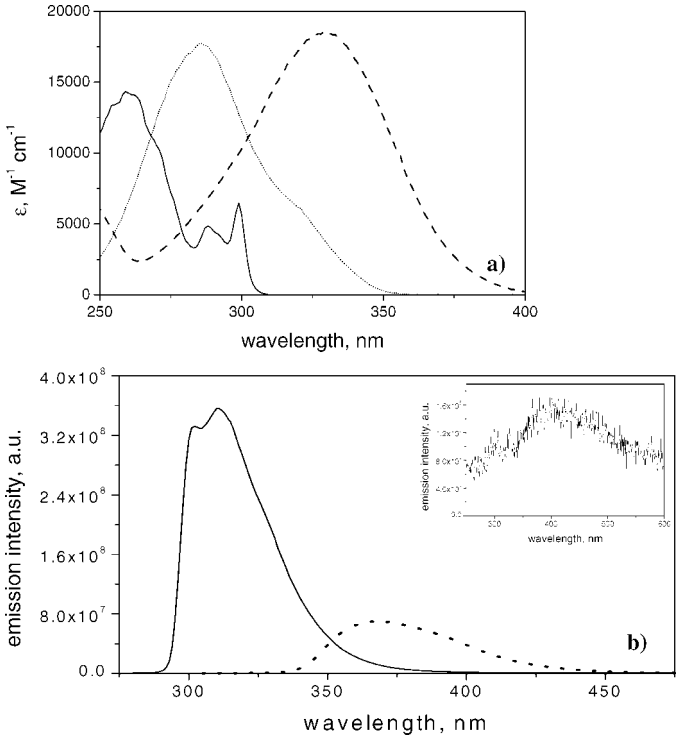

FIG. 2. (**a**) Absorption spectrum of fluorene (solid line) and its derivatives 2-NO_2F (dashed line) and 2-NH_2F (dotted line) in MeOH. (**b**) Emission spectrum of fluorene (solid line) and its derivative 2-NH_2F (dotted line) in MeOH, fluorescence excitation, and emission wavelengths are the same as in Table I (for additional spectra see the Supplemental Material); $\lambda_{exc} = 260$ for fluorene, $\lambda_{exc} = 285$ for 2-aminofluorene. (**Inset**) Emission spectrum of 2-nitrofluorene in MeOH.; $\lambda_{exc} = 230$.

these compounds to produce ROS; it was also demonstrated that these characteristics can be directly associated with their toxicity.[14]

Polycyclic aromatic hydrocarbons and their derivatives have closely similar structures (Fig. 1). For a general example, the absorbance and emission spectra of fluorene and its derivatives in MeOH are shown in Figs. 2a and 2b. The absorption spectrum of F is extended to about 310 nm and shows a marked absorption between 260 nm and 300 nm. For NO_2-F only, a prominent maximum at 330 nm is noted, whereas the NH_2-F shows one band centered at 285 nm (molar absorption coefficient $\varepsilon = 17\,700\ M^{-1}cm^{-1}$), with a tail extending to 350 nm. The fluorescence of the nitro derivative is negligible (see inset, Fig. 2b), with a very weak emission at about 410 nm. Conversely, amino and parent PAH are strongly fluorescent and exhibit emission maxima at 370 nm and at about 310 nm, respectively. As shown in Tables II and III and the Supplemental Material,† analogous spectral differences between the parent and derivatives are generally observed for the other derivatives and parent PAHs; in particular, the absorption-spectrum shift towards longer wavelengths of amino and nitro derivatives versus PAHs reflects the differences in the electronegativity.[10] While most PAH compounds are markedly fluorescent in MeOH (Table III), the strong electron-withdrawing effect of the nitro group renders NO_2-PAHs nonfluorescent. Conversely, if the NO_2 functionality is reduced to the NHOH or NH_2 functionality, the resulting compounds will again be fluorescent (Table III). The reported fluorescence quantum yields were determined using the value of Naproxen fluorescence in methanol as a standard ($\lambda = 0.53$, $\lambda_{exc} = 312$ nm).[16]

Significant fluorescence of NO_2-PAHs is present only in two compounds, NO_2-P and NO_2-BaP, the emission being slightly weaker with respect to the corresponding amino derivative (see Supplemental Material). Some authors explain this unexpected fluorescence with the assumption that the nitro substitution (in some positions of terminal rings) increases the solubility of the compound, yet does not affect the planarity of the chromophore or the strength of the transition (which are requirements of emission strength). For these reasons, these substituted compounds show characteristics very similar to those of the unsubstituted compounds.[17]

Taking into account these experimental results, detection of NO_2-PAHs by liquid chromatography has been accomplished

† Supplemental Material is available on-line in the electronic version of the journal (http://www.s-a-s.org).

TABLE II. Absorption parameters of PAH and derivatives in MeOH.

PAH[a]	λ, nm; ε, M^{-1} cm^{-1} × 10^{-3}	NO_2-PAH	λ, nm; ε, M^{-1} cm^{-1} × 10^{-3}	NH_2-PAH	λ, nm; ε, M^{-1} cm^{-1} × 10^{-3}
P	333(p), 34.8; 318(p), 21.5 305(p), 8.69; 271(p), 32.5	1-NO_2P	397(p), 11.3; 373(p), 11.5 350(s), 9.65; 288(p), 15.1	1-NH_2P	242(p), 36.1; 282(p), 20.5 356(p), 14.5; 402(s), 8.02
F	260(p), 15.8; 288(p), 4.86 299(p), 6.47	2-NO_2F	329(p), 18.6	2-NH_2F	285(p), 17.7; 321(s), 6.08
PAN	292(p), 7.01; 280(p), 6.07 273(p), 7.73; 250(p), 39.5	9-NO_2PAN	339(p), 1.46; 291(s), 2.18 268(s), 4.82; 247(p), 12.1	9-NH_2PAN	365(s), 0.43; 314(p), 3.64 250(p), 17.8
		3-NO_2PAN	340(p), 9.17; 299(p), 5.20 262(s), 22.9	3-NH_2PAN	250(p), 33.4; 282(p), 7.87 307(p), 6.78; 360(p), 1.00 250(p), 47.0
BPH	245(p), 13.4	3-NO_2BPH	247(p), 19.8; 315(s), 1.19	3-NH_2BPH	300(p) 2.69
BaAN	286(p), 55.8; 325(p), 3.67 340(p), 4.13; 356(p), 2.74	7-NO_2BaAN	386(p), 2.96; 366(p), 4.54; 250(p), 47.0 286(p), 43.5; 277(p), 46.4	7-NH_2BaAN	258(p), 11.8; 300(p), 12.9 390(p), 2.13; 400(p), 2.22
BaP	402(p), 1.81; 382(p), 16.6 363(p), 15.0; 346(p), 7.60	6-NO_2BaP	430(p), 48.0; 369(p), 68.8 286(p), 136; 298(p), 131	6-NH_2BaP	258(p), 27.6; 308(p), 19.0 296(p), 21.4; 423(p), 10.6
CHR	318(p), 9.71; 305(p), 8.36 295(p), 3.97; 266(p), 32.9	6-NO_2CHR	288(s), 16.2; 365(p), 6.76	6-NH_2CHR	273(p), 65.4; 337(p), 13.5
AN	374(p), 5.95; 356(p), 6.48 338(p), 4.27; 322(p), 2.25	9-NO_2AN	470(p), 0.75; 324(s), 3.22 270(s), 15.5	9-NH_2AN	252(s), 1.45; 264(p), 2.76
	360(p), 16.1; 345(p), 17.6 278(p), 49.7; 245(p), 54.3	3-NO_2FA	381(p), 12.7; 345(p), 9.94 302(s), 8.24	3-NH_2FA	301(p), 19.6; 325(p), 7.72 366(p), 5.93; 405(p), 5.83

[a] Ref. 20.

TABLE III. Fluorescence detector setup and emission quantum yields of PAHs and their derivatives in MeOH.

PAH	λ_{exc}	λ_{em}	Φ_F	NO_2-PAH	λ_{exc}	λ_{em}	Φ_F[a]	NH_2-PAH	λ_{exc}	λ_{em}	Φ_F
P[b]	338	372	0.53	1-NO_2P	384	406	0.45	1-NH_2P	355	430	0.61
F[b]	260	340	0.68	2-NO_2F.	230	410	0.023	2-NH_2F.	285	370	0.75
PAN[b]	255	347	0.12	9-NO_2PAN	250	390	0.0074	9-NH_2PAN	310	440	0.32
PAN[b]	255	347	0.12	3-NO_2PAN	245	383	0.0046	3-NH_2PAN	307	405	0.34
BPH[c]	246	310	0.18	3-NO_2BPH	250	323	0.0069	3-NH_2BPH[h]	300	400	0.30
BaAN[d]	286	384	0.20	7-NO_2BaAN	333	414	0.0004	7-NH_2BaAN	400	480	0.45
BaP[e]	300	400	0.32	6-NO_2BaP	300	460	0.4	6-NH_2BaP	423	520	0.53
CHR[d]	270	380	0.17	6-NO_2CHR	260	380	0.0011	6-NH_2CHR	337	440	0.34
AN[f]	265	390	0.27	9-NO_2AN	250	424	0.061	9-NH_2AN	262	510	0.61
FA[g]	360	400	0.30	3-NO_2FA	254	440	0.0078	3-NH_2FA	410	530	0.53

[a] The fluorescence quantum yields were determined using the value of Naproxen fluorescence in methanol as a standard ($\Phi = 0.53$, $\lambda_{exc} = 312$ nm).[16]
[b] Ref. 21.
[c] Ref. 22.
[d] Ref. 23.
[e] Ref. 17.
[f] Ref. 24.
[g] Ref. 25.
[h] Ref. 13.

after reduction by electrochemical means. A coulometric detector is operated in the reductive mode, followed by fluorometric detection of the amine derivative; additional selectivity in this case can be gained by acquiring a full fluorescence spectrum of the elute.

Chemical reduction of NO_2-PAH in NH_2-PAH is a frequently used method for quantification of analytical derivatives; for this reason the evaluation of the reduction efficiencies of NO_2-PAHs is important. As shown in Table I, the reduction yield of nitro to amino derivatives is almost complete. These data were verified by HPLC (Table I): identification of reactants, as well as identification of products, was carried out through ESI-MS measurements. Because of possible ESI ion suppression, possibly due to HPLC buffer, and in order to verify reduction, some amino standards were also utilized to confirm the results obtained.

In the case of PAH analysis, fluorescence setup was usually made with excitation wavelengths in the UV region due to higher emission intensities. This should be avoided when analyzing derivatives in order to avoid overlapping absorption. This results in a better differentiation between derivatives and parent compounds.

The need for an accurate and unambiguous qualitative and quantitative determination of PAHs and their nitro and amino derivatives, either alone or in mixture, is justified by the fact that in the last decades the presence of these substances in organisms was reported. Indeed, the main pathway of degradation for NO_2-PAHs seems to occur through metabolic reduction in amino derivatives; in some cases these latter were found in urine.[11] It is noteworthy that the toxicity of the amino derivatives increases with the decrease of their potential.[18] In Table III some $E_{1/2}$ values are shown. Indeed, by coupling the electronegativity of nitro derivatives together with their reduction potential, we can have some confidence about the order of NO_2-PAHs toxicity, as reported by some authors.[18]

CONCLUSION

The correlation between the effect of NO_2-PAH metabolic reduction with the ability of these compounds to produce ROS has been reported; it has also been demonstrated that these characteristics can be directly associated with their toxicity.[14]

The spectroscopic analysis showed that the nitro group of PAH derivatives quenches fluorescence. Indeed, the NO_2-PAHs are not fluorescent except for a few compounds.

Nevertheless, HPLC/FL of NO_2-PAHs after reductive modification is relatively sensitive, cheap, and rapid but requires detailed knowledge of the sample. Such an analysis ideally allows evaluation of nonpolar (PAHs) and polar (PAH derivatives) compounds in one run. Thus, the measurement of amino derivatives via ESI-MS allows information to be acquired about structure, thereby obtaining unequivocal product identification. The HPLC/FL/MS is a very efficient method in a wide range of experimental conditions for measurement of environmental pollutants.

SUPPLEMENTAL MATERIAL

The Supplemental Material mentioned in the text, including a full layout of the spectra of the PAH derivatives, is available on-line in the electronic version of the journal (htt://www.s-a-s.org).

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