Molecular changes during natural biopolymer ageing − The case of shellac

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ABSTRACT

Shellac is derived from the resinous secretion of the lac insect (Kerria lacca), mainly found in India. This biopolymer is made of natural single and polyesters of aliphatic acids and sesquiterpenoid acids. Ageing was investigated through interdisciplinary approaches to understand the behaviour and the interactions of macromolecules in the biological system. During ageing the viscoelastic properties of the polymer evolved in the sense of crosslinking of the macromolecules. The fate of the shellac backbone molecules was assessed. It was found that hydroxy fatty acid such as aleuritic acid could be a factor in the changes of the polymer consistency. Aleuritic acid could be freed from the polymer during its ageing after de-esterification and could participate through hydrogen bonding in the biopolymer crosslinking observed by melt rheology.

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1. Introduction

The legislative authorities, in June 2007, restricted and prohibited the use of some chemical substances for industrial purposes in the REACH program. This increasing environmental consciousness led researchers to focus on molecules with a global sustainability. In this sense, biopolymers are eco-friendly compounds with a great interest. They are encountered in vegetable or animal media through three main classes: proteins, lipids and polysaccharides. Starch and cellulose are examples of biopolymers taken for applications in industry of biobased polymers to substitute petrochemical polymers [1].

Shellac is another biopolymer where more and more concern for sustainable applications are taken. Shellac is a resin of animal origin (Laccifer lacca, Coccus lacca) and has a considerable social economic significance for Asiatic countries such as India, Thailand and Myanmar. In ancient times, shellac was used as a protective agent for wooden furniture and paintings. It confers glossy properties, protects from chemical reactions and mechanical abrasion. Even if shellac has been replaced by better performing synthetic varnishes composed of ketone or acrylic resins dissolved in mineral spirits, its use is becoming larger and larger. Shellac is an eco-friendly and biodegradable polymer [2]. Shellac is non toxic and physiologically harmless: it is listed as GRAS (Generally Recognized As Safe) by the FDA. Shellac has been used in pharmaceuticals for its excellent film forming and acid resistance properties [3]. In food industries, shellac is an additive, known as E904. Lots of research was done for the modification or enhancement of shellac chemical and mechanical stability [4,5] by using plasticizer such as triethyl citrate [6] or by adding acrylic resin in order to make a more eco-friendly coating. These studies open a new way of consumption of shellac. It is also used in the design of new biomaterials in the area of drug eluting stents, for its coating and drug release properties [7]. However the major applications of shellac are in cultural heritage since the resin is present in many art and archaeological objects. Shellac was used as a varnish coating on some famous paintings [8]. Shellac is also used as a thermoplastic resin to consolidate deteriorated archaeological wood [9].

Characterization of the material is not easy due to the macromolecular nature of shellac. However it was partly overcome by preliminary saponification or pyrolysis pre-treatments which transform esters into more volatile compounds which can be detected by gas chromatography coupled with mass spectrometry (GC−MS) as previously described [10].

From a chemical point of view, shellac is a natural polymer which is a mixture of two resins: 70% hard resin (insoluble in ether) designated as the "pure lac resin" and 30% soft resin (soluble in ether) [11,12]. It contains around 67.9% carbon, 9.1% hydrogen and 23% oxygen [13]. Shellac is mainly made of single and polyesters of aliphatic acids and sesquiterpenoid acids. These natural compounds are of great interest in stress-induced defence and they are present in excretion of several animals such as butterflies [14].

Concerning shellac behaviour upon time, much less information has been found, except that aldehyde groups are very easily oxidized and transformed into carboxylic acid groups and that it certainly becomes less alcohol-soluble, meaning that the free hydroxyl groups are less and less available [11,15]. New high viscosity polymeric dispersion had to be designed in order to properly clean ancient objects varnished with shellac without damage [16]. For this purpose, study of aged shellac is necessary to better understand its mechanical properties and interactions towards its environment.

The present study focus on the photoageing patterns of shellac under simulated UV–visible light and natural sunlight. Macromolecular change during irradiation was measured by means of melt rheology. Molecular changes were evaluated by liquid and gas chromatography.

2. Experimental section

2.1. Material

The material investigated in this study was wax-free shellac from Sigma Aldrich (product 78471-250 G, batch BCBC2696). The wax was extracted after dissolution of sticklac in ethanol and evaporation of ethanol. Two others type of shellac were compared in this study: luna lac and elephant lac. Both were supplied by the *Laboratoire Chimie Appliquée à l'Art et à l'Archéologie*, in Avignon.

2.2. Film preparation

Thin films were obtained by pressing the wax-free shellac between Teflon-coated sheets at 100 °C for 1 min, under a pressure of 170 bars. Thickness of shellac films was homogeneous to (100 ± 20) μm.

2.3. Photoageing

Shellac films were irradiated in a photoageing device equipped with a mercury-vapour lamp (Novalamp RVC 400 W) which emits spectral rays above 295 nm and a total flux in the range of 2.6 mW cm^{-2}. This device has been designed in order to accelerate the photodegradation of polymers in artificial conditions which are relevant to natural ageing. Five different artificially exposure times are presented: 5 h (T5), 10 h (T10), 15 h (T15) and 20 h (T20). Shellac was also exposed to natural conditions in order to evaluate the effect of direct sunlight during 3 days (T1), 6 days (T2) and 9 days (T3).

Shellac films were introduced into a closed Pyrex reactor with a volume of 335 mL. Temperature inside the reactor was approximately constant to 40 °C.

Films were analysed after various exposure times. One part of the film was kept for testing viscous and elastic properties at the molten state and the other part was used for LC/MS and GC/MS analysis.

2.4. Characterization techniques

Melt rheological experiments were carried, in oscillatory mode, on a rotational rheometer (ARES, TA, USA) equipped with a parallel plate (8 mm diameter). All samples were measured at 80 °C with 1 mm gap distance. The imposed oscillatory shear stress amplitude was tested to validate the measurements inside the linear viscoelastic domain.

Concerning viscoelastic material, there is no single parameter that can be used to characterize the stress strain relationship. The complex dynamic modulus (G^*) is resolved into two components using complex notation (1) and the complex dynamic viscosity (η^*) is defined as complex notation (2). The zero shear viscosity η_0 can be obtained by the equation (3).

$$G^* = G' + iG'' \tag{1}$$

$$\eta^* = G^*/(i\omega) = \eta' - i\eta'' \tag{2}$$

$$\left|\eta^*\right|_{\omega \to 0} = \eta'_{\omega \to 0} = \eta_0 \tag{3}$$

G' is defined as the storage modulus (or elastic modulus) and G'' is the loss modulus (or viscous modulus). η' is called the loss viscosity and η' is the storage viscosity. ω is the shear frequency submitted to the material.

The LC/MS system consisted of a Waters® Alliance® 2695 high-performance liquid chromatography (HPLC) set-up (Waters SA) equipped a quadrupole time-of-flight (micro-QTOF) mass spectrometer (Micromass). The operating cone voltage was set to 35 eV for (ESI +) and (ESI −). Shellac samples were dissolved in methanol and were chromatographed on a reversed-phase column (C18 XTerra® MS, 3.5 μm, 2.1 mm × 100 mm; Water SA) at a flow rate of 0,2 mL min^{-1}. The mobile phase was composed of water with 0.1% formic acid (A) and acetonitrile (B). Linear gradient elution was performed as follows, A/B: 95/5–5/95 in 15 min, 5/95 for 10 min and 5/95–95/5 in 10 min. Sample injection volume was 10 μL. Methanol was used as a solvent for injection for both shellac and aleuritic acid because all samples were totally solubilised.

GC/MS experiment were led according to the previously described protocol[11]: 15 mg of grinded sample was hydrolysed by adding 1 mL of a KOH solution in binary mixture of methanol and water (10% KOH/40% of MeOH/50% Water in weight) at 60 °C for 4 h. Solution was, then, acidified with hydrochloric acid (10 M) and organic molecules were extracted 3 times with 1 mL of diethyl ether. The 3 extracts were combined and solvent was evaporated to dryness under a stream of nitrogen. Finally, the sample was trimethylsilylated by reaction with 500 μL of pyridine, 450 μL of HMDS and 300 μL of TMSCl at room temperature for 30 min. The trimethylsilylated extract was dried with a stream of nitrogen and dissolved in 1 mL of diethyl ether and analysed by GC/MS.

GC/MS analysis were performed using a Varian Saturn 3900 gas chromatograph equipped with a Varian 1177 injector and coupled with a Varian 2100 T ion trap mass spectrometer (Varian, Walnut Creek, CA, USA). The GC column was a fused silica capillary column Varian CP-Sil 8 CB low bleed/MS (30 m length × 0.25 mm i.d. × 0.25 μm film thickness). Molecular component were eluted using helium as carrier gas at a constant flow of 1 mL min^{-1} with the following temperature program of the oven: 50 °C for 2 min, 50–250 °C at 8 °C min^{-1}, 250–350 °C at 3 °C min^{-1} 1 μL of each sample was injected with a splitting ratio of 1:20 and injector temperature was set to 250 °C. Mass spectra were record in electron impact (EI) with an electron ionization voltage of 70 eV, an ionization time of 25,000 μs and a mass range of 40–650 m/z. Transfer line, ion trap and manifold temperatures were respectively of 300 °C, 200 °C and 50 °C.
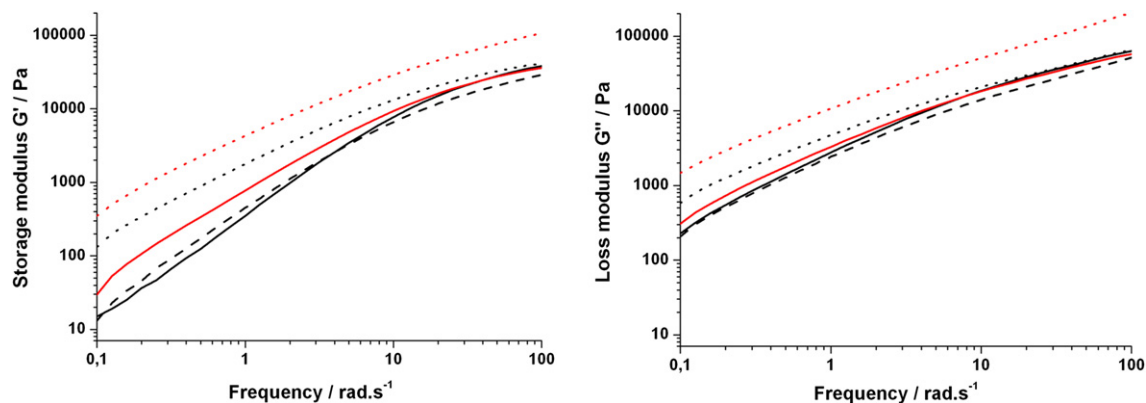
Fig. 1. Storage and loss moduli of studied shellac (——), elephant lac (– – –), luna lac (· · ·), naturally aged shellac (——) and artificially aged shellac (· · ·).

3. Results and discussion

3.1. Rheological behaviour upon shellac photoageing

Shellac presents similar mechanical patterns compared to other biopolymers such as starch or cellulose [17,18]. The storage modulus increases from 10 to 10,000 Pa with increasing shear frequency from 0.1 to 100 rad s^{-1}. By comparison to other types of shellac, it has the same melt rheological behaviour as the elephant lac. However, these are quite different compared to the luna lac (Fig. 1) which presents higher storage and loss moduli. The luna lac is chemically and mechanically different with molecular weight and viscosity bigger than the studied one.

Both naturally and artificially aged shellac present higher values of G' and G'' compared to the pristine one with a shear frequency comprised between 0.1 and 10 rad s^{-1}. From 10 to 100 rad s^{-1}, values of G' and G'' reach the rubbery plateau for the naturally exposed sample, whereas the artificially ones are upper.

These increase of storage and loss moduli are representative of a crosslinking of the polyester matrix. This formation of the three dimension networks inside the biopolymer can be quantified thanks to the zero shear viscosity η_0 in the Cole Cole plot (Fig. 2). η_0 is calculated by an extrapolation of the semi-circle in the abscissa [19]. At initial state, shellac presents an η_0 equal to 3100 Pa s η_0 increases rapidly in function of artificially exposure time and at a lesser extent of naturally exposure time. After nine days (T3) of

natural sunlight, the Cole Cole plot is still a semi-circle with η_0 equal to 4650 Pa s. This value is still lower compared to the value of the 5 h (T5) artificially irradiated shellac ($\eta_0 = 7020$ Pa s). T5 experiment was done without and with a recombined air flow to test the oxidative reactions with oxygen but little difference was observed ($\eta_0 = 5220$ Pa s). The zero shear viscosity η_0 increases to 17,120 Pa s and 27,350 Pa s respectively after 10 h (T10) and 15 h (T15) of artificial irradiation. After 20 h of artificial light, the Cole Cole plot is no more a semi-circle but a straight line, meaning that the macromolecules are present in a gel-like structure.

Concerning the kinetic of crosslinking shellac, it was found a good exponential fit of η_0 and artificial exposure time from T0 to T15, as follows $\eta_0 = Ae^{k_{cr}t}$, with $A = \eta_0$ at initial time (T0) and $k_{cr} = (4.03 \pm 0.50)10^{-5}$ s^{-1}.

The kinetics of crosslinking are different from natural to artificial irradiations. First of all the total flux of the two sources are different and the wavelengths responsible for photodegradation are not the same.

3.2. Molecular changes during shellac photoageing

Some aliphatic and sesquiterpenoid constituents of the pristine shellac were identified by their total ion current (TICs) traces. Positive and negative ion modes were used in order to get their molecular weight. Hydroxy aliphatic compounds were identified at time 10.5 min and 14.8 min, respectively as aleuritic acid and butolic acid. Concerning the sesquiterpenoid constituents, their
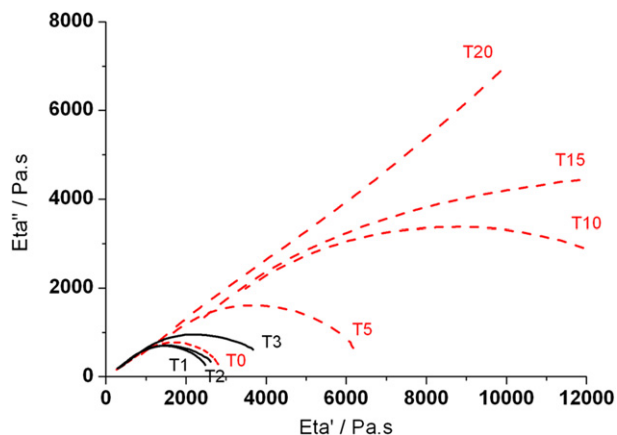

Fig. 2. Cole Cole plots of naturally (full line) and artificially (dashed red line) irradiated shellac samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Peak assignment and mass spectra features for shellac samples detected by HPLC-ESI-MS, using the positive ion mode.

| Compound	Retention time (min)	M_w	Characteristic m/z
Aleuritic acid	10.5	304	251, 269, 287, 305, 327, 343, 610
Ester derived of aleuritic acid	11.5	582	251, 269, 287, 305, 311, 343, 549, 567, 583, 605, 621, 894
Ester derived of jalaric acid and aleuritic acid	12.2	566	251, 269, 287, 305, 549, 567, 589, 605, 870
Ester derived of aleuritic acid	13.7	532	229, 269, 289, 305, 497, 515, 531, 533, 551, 568, 573, 589, 845
Butolic acid	14.8	244	191, 209, 227, 245
Palmitic acid	19.7	256	257, 297, 511
Laksholic acid and Epilaksholic acid	19.9	282	283, 564
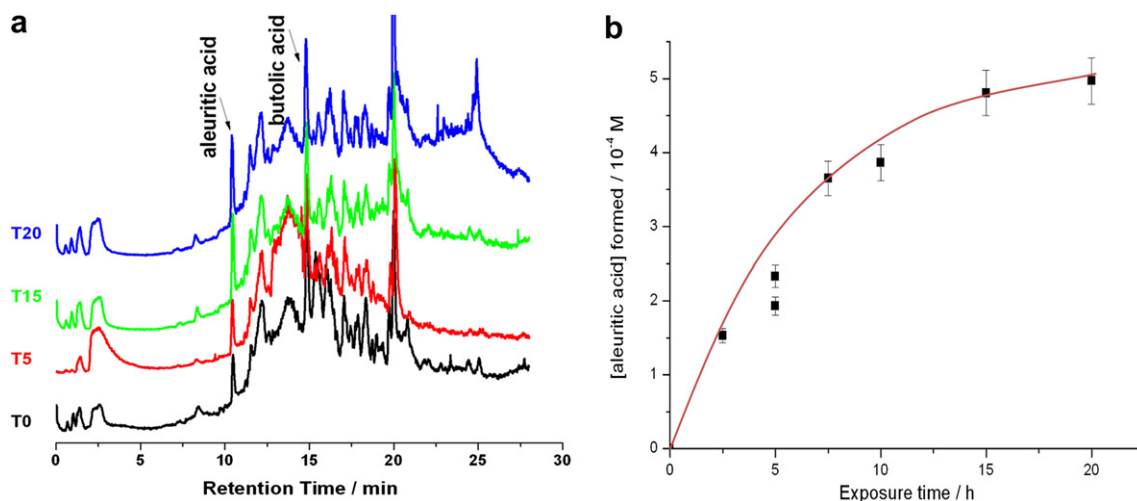
Fig. 3. Total ion chromatogram traces obtained by HPLC-MS, using the positive ion mode. (a) And kinetic of formation of aleuritic acid at different exposure times (b).

detection were quite inappropriate with this technique. However, the molecule retained at 12.2 min was attributed to the single ester made of jalaric acid and aleuritic acid ($M_w = 566$ g mol^{-1}). Mass spectra features of different compounds are given in Table 1.

Much less difference was observed between all the TICs at the various exposure times by HPLC-MS (Fig. 3a), except for aleuritic acid peak. Concerning HPLC-MS, an increase of the aleuritic peak signal was observed. This increase was quantified by an external calibration of aleuritic acid. This increase was well fitted ($R^2 = 0.98$) by [aleuritic acid] $= K(1 - e^{k_{app}t})$, with K corresponding to the initial concentration of aleuritic acid: $(4.32 \pm 0.30)0.10^{-4}$ mol L^{-1} and $k_{app} = (3.10 \pm 0.75)0.10^{-5}$ s^{-1} (Fig. 3b). Butolic acid and esters derived from aleuritic acid present no change during shellac photoageing with this technique. Butolic acid has already showed stability under pyrolysis conditions and has been proposed as a marker for shellac resin [10,15]. From the kinetic study the same order of magnitude of $3-4.10^{-5}$ s^{-1} of crosslinking rate constant k_{cr}

and aleuritic acid production rate constant k_{app} can be noted. These two phenomena are occurring at the same time scale and it is possible that the increase in aleuritic acid after de-esterification from the backbone of shellac could lead directly to a three dimensional re-organisation of the biopolymer, through weak bonds with the hydroxyl groups. The involvement of other hydroxyl fatty acids in the three dimension structure cannot be excluded.

In order to obtain additional qualitative information, samples were submitted to alkaline hydrolysis followed by GC–MS analysis (Fig. 4). Different chemical components of shellac were identified, their mass spectra feature were similar to those previously described in literature [10]. First of all, these experiments enable the detection of aleuritic acid ($t = 34.53$ min) and butolic acid ($t = 24.38$ min) thanks to their mass spectra (Fig. S4). Moreover, this permit to detect sesquiterpenoid component as epilaccijalaric acid ($t = 26.873$ min), laccishellolic acid ($t = 27.600$ min) and derivate compound, jalaric acid ($t = 28.123$ min) and derivate compounds.
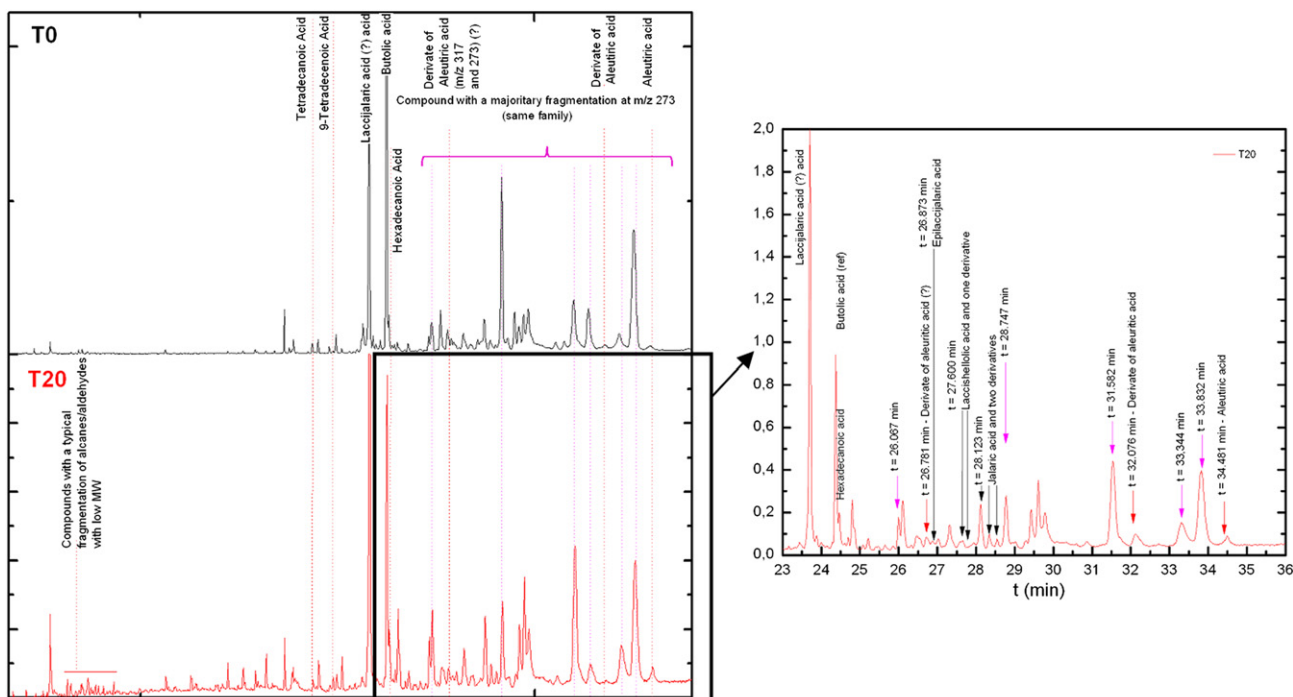

Fig. 4. Total ion chromatogram traces obtained by GC–MS of pristine shellac (T0) and artificially photoaged shellac (T20), using the positive ion mode.

During the photoageing experiment, it's worth noting the formation of several unknown molecules with a similar fragmentation of aleuritic acid, especially with a strong fragment with 273 Da nominal mass (Fig. S5). In the aleuritic acid, this fragment ($m/z = 273$) is formed after the cleavage of the C—C bond following the hydroxylated carbon. This has already been observed on similar fatty acid molecules [20]. Thus, these components could be different hydroxylated fatty acids with, at least, one hydroxylation on the carbon 9. Such molecules could come from different reaction of hydroxylation of fatty acids [15,21].

Therefore, the aleuritic acid contribution to the photoageing mechanism of the polyester seems to be clearer. Its apparition takes place with the same kinetic compared to that of the crosslinking of shellac. The hydroxy unsaturated aliphatic acid could be released from the sesquiterpenoid acid of the polymer matrix through de-esterification. Hydroxyl groups are free for forming linkages to surrounding molecules. In a plant cutin mimetic polymer, it was proved that aleuritic acid could also change the mechanical properties and initiates the polymer network through hydrogen bonding [22]. As cutin is a biopolymer with some similarities to shellac, one could expect that aleuritic acid act as a precursor of the observed crosslinking.

4. Conclusions

Our main finding is the crosslinking of shellac during its natural and artificial ageing. The mechanism has been found to be well correlated to one compound of the natural resin: aleuritic acid. Shellac crosslinking and aleuritic acid formation are occurring at the same time scale during photoageing. This hydroxy aliphatic fatty seemed to be the central connection of the polyester matrix during the formation of the three dimensional macromolecular networks. A group of molecules with the same mass fragmentation than aleuritic acid was found and two of them were totally inexistent initially. They are still not identified but could be in the future and be used as possible interesting degradation markers for shellac analysis in other matrix. This discovery will have important applications in the biopolymer or biosourced polymer design and in the understanding of biopolymer molecular evolution towards ageing.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.polymdegradstab.2012.03.024.

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