Determination of Wool Wax in Raw Wool by Raman Spectroscopy

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A Raman spectroscopic method for the determination of wool wax content in raw wool has been developed. The analyses were performed on 250-mg wool samples by using a spectral component band resolution method. The method has a moderate sample throughput rate, is nondestructive and does not require the use of solvents. Samples ranging between 0.5 and 31% (w/w) wool wax were analyzed. The results obtained were compared with those obtained by a solvent extraction method. If the nonextractable lipid content of the wool is considered, the results of the Raman spectral and extraction methods were in excellent agreement. From a practical standpoint, the minimum detection limit of the method is 3% wax. In general, the precision of the Raman spectroscopic method was better than that obtained for the extraction method.

KEY WORDS: FT-Raman spectroscopy, quantitation, raw wool, wool wax.

Wool wax is the greasy secretion produced by the sebaceous glands of sheep. In the literature it is also commonly referred to as wool grease. It is a complex material that contains high-molecular weight esters, together with some free alcohols and acids (1–3). The wax content of raw wool samples is one of the fundamental parameters that ultimately determines the selling price of a bale of wool. For Australian raw wool, the wax content can range from over 30% to as low as 5%. From the standpoint of a research and development laboratory, it is also important to be able to quantitate the wax content of a raw wool sample on a rapid basis. In particular, when the wool is from a single pen-grown sheep, it is important that as little fleece as possible is wasted during the analysis.

Currently utilized methods for the determination of wool wax in wool include solvent extraction (4,5) and near infrared analysis (NIRA) (6,7). A wide-line proton magnetic resonance method has also been reported (8). Solvent extraction procedures require large quantities of solvents, many of which, such as dichloromethane, are toxic and considered environmentally unfriendly. Typically, analysis by this method takes six to nine hours and requires a sample mass of 10 g. An NIRA method is commonly used for the determination of residual wool wax after scouring (6). This method has an analysis rate of eleven tests per hour and requires a 10-g sample. Attempts to determine wax levels greater than 1% with this method have not been successful. The NIRA method for the measurement of the wax content of raw wool reported by Connell and Norris (7) requires a sample mass of 2 g and is considered nondestructive by the authors, even though the wool has to be powdered prior to analysis. The calibration procedure for the NIRA methods is quite laborious and involves measurement by both the reflectance and extraction methods of well over 100 reference samples spanning the range of analytical interest. The calibration is thus time-consuming, requires the use of large amounts of solvent, and such calibrations are generally not transferable from one instrument to another.

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Recent application of Fourier transform (FT)-Raman spectroscopy to the study of wool has succeeded in obtaining good-quality data that are rich in spectral information with little or no sample preparation. The problems of fluorescence and sample damage due to high-beam power encountered with traditional laser Raman spectroscopy are no longer significant drawbacks (9,10). Raman spectroscopic studies on lipids have shown that the vibrational C-H stretching region between 2700 and 3100 cm^{-1} is highly sensitive to hydrocarbon chain structure (11–13). This paper describes an accurate and relatively quick method, based on Raman component band analysis of this spectral region, for the determination of wool wax in raw wool samples.

EXPERIMENTAL PROCEDURES

Raman spectra. FT-Raman spectra were obtained with an Bruker IFS 88 interferometer (Bruker Analytische Messtechnik GMBH, Karlsruhe, Germany) with a FRA 106 Raman attachment. The Raman attachment was equipped with an Adlas Nd:YAG laser (Karlsruhe, Germany) operating at 1.064 microns. A laser power of 210 mW at the sample and 180° sampling geometry were utilized. Data acquisition was performed with Bruker OPUS software (version 1.4).

During the initial method development, FT-Raman spectra were collected at a resolution of 4 cm^{-1} and a scanner velocity of 0.17 cm/s. Noise reduction was achieved by averaging 250 scans, which resulted in a scan time of approximately 20 min. The sample throughput was later optimized by increasing the scanner velocity to 0.53 cm/s and reducing the number of scans to 150. These changes resulted in the reduction of scan time to 4 min. A Norton-Beer strong apodization function was used, and all spectra were corrected for instrumental effects by applying the Raman correction function provided by the instrument manufacturer.

The fiber cell used throughout this study has been described elsewhere (10). For each spectroscopic wax determination, about 250 mg of wool was used. Three spectra were recorded from different areas of each sample. Component band analysis work was performed with SpectraCalc Arithmetic software version A2.12 (Galactic Industries Corporation, Salem, NH). The chi-squared values obtained for the band fits were typically less than 0.06.

Raw wool and wax samples. Raw wool samples were obtained from the fleece of two pen-grown merino sheep (CSIRO Div. of Animal Production, Prospect, New South Wales). Samples were acquired from the front, middle and back sections of the fleece. Scoured merino wool samples and unprocessed wool wax were obtained from several commercial SiroScour plants.

Methods. Laboratory samples of Merino wool with wax contents of 10% or less were prepared from samples of scoured wool and unprocessed wool wax. A known quantity of scoured wool was placed inside a round-bottom flask. The required amount of wax was dissolved in a volume of isopropyl alcohol (AR-grade) that was just enough to wet the fibers (without any excess solution

remaining inside the flask). The alcohol/wax solution was then added to the flask, and the fibers were then air-dried.

Each of the wool samples were quantitatively analyzed for wax in triplicate by using a modified solvent extraction procedure based on the Australian Standard AS2001.3.4 (4). In the extraction procedure, 4-g samples of raw wool were conditioned to constant weight at 65% relative humidity and 20°C. The test specimens of wool were then extracted with 60 mL dichloromethane (AR-grade) under reflux for 15 min in a Tecator Soxtec System HT 1043 Extraction Unit (Tecator AB, Höganäs, Sweden). The condensed solvent was allowed to rinse through the test specimen for 30 min to remove any residual soluble matter, and the solvent was then allowed to evaporate. The percentage of solvent-extractable matter was calculated based on the oven-dry mass of the extracted specimen.

RESULTS AND DISCUSSION

The Raman spectra of raw Merino wool containing approximately 30% wool wax (Fig. 1a), and crude wool wax (Fig. 1b) indicate the quality of the data from which our wax quantitations are derived. Figure 2 presents the 2700 to 3200 cm^{-1} regions of these spectra, scaled such that the wool wax spectrum (b) represents the 30% wool wax component of the raw wool spectrum (a). The spectrum obtained from scoured Merino wool is shown as Figure 2c. It is clear from this representation that it should be feasible to estimate the wax content of raw wool by merely comparing the areas of the wax and raw wool bands in this spectral region. Because the bands of the wool and wool wax overlap in this spectral region, it is necessary to first characterize the band components of the scoured wool and the wax separately before analysis of the raw wool band complex can be attempted.

For wool, the 2800 to 3100 cm^{-1} spectral region is rich in C-H stretching fundamentals and the bending overtones of amino groups. These vibrations arise largely from the wool polypeptide skeleton and amino acid side chains.

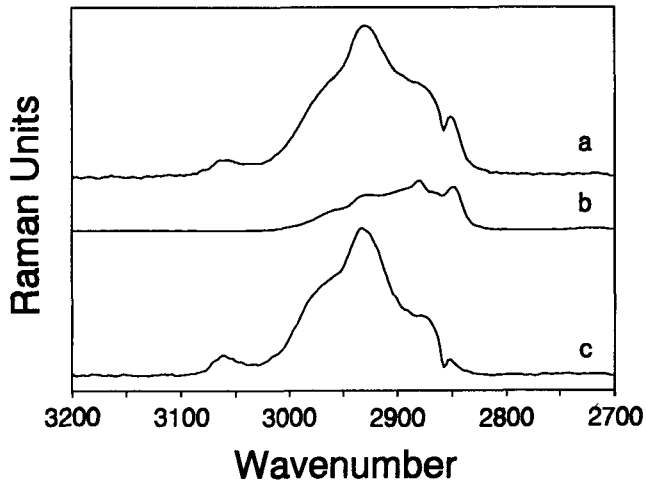

FIG. 2. The 2700 to 3200 cm^{-1} region of the Fourier transform-Raman spectra obtained from (a) raw Merino wool containing approximately 30% (w/w) wax, (b) wool wax scaled so that the band area is approximately 30% that of the raw wool band area and (c) scoured Merino wool.

There is also a minor component from the structural lipid content of the wool that constitutes 0.8–1% of the total fiber mass (14). In contrast, the wool wax spectrum exhibits Raman lines that are mainly associated with aliphatic chains. This spectrum would be similar to that expected from the lipid component of wool.

In the Raman spectra obtained from both the scoured wool and the wool wax (Fig. 2), the CH_2 asymmetric stretching modes near 2930 cm^{-1} dominate the complex broad band structures. Shoulders on both sides of this band are also evident. The first overtone of the C-N-H bending mode is observed in the spectrum of the scoured wool near 3060 cm^{-1}. The deconvolution of the complex bands for the scoured wool and wool wax samples are shown as Figures 3 and 4, respectively. The band fit

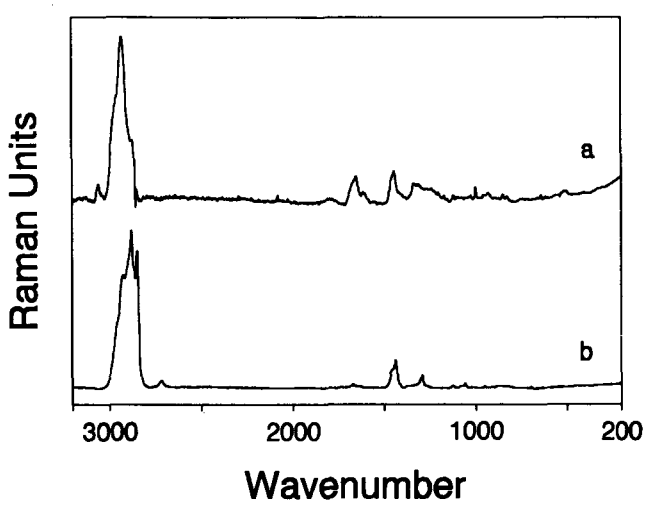

FIG. 1. Fourier transform-Raman spectra of (a) raw Merino wool containing approximately 30% wool wax and (b) wool wax.

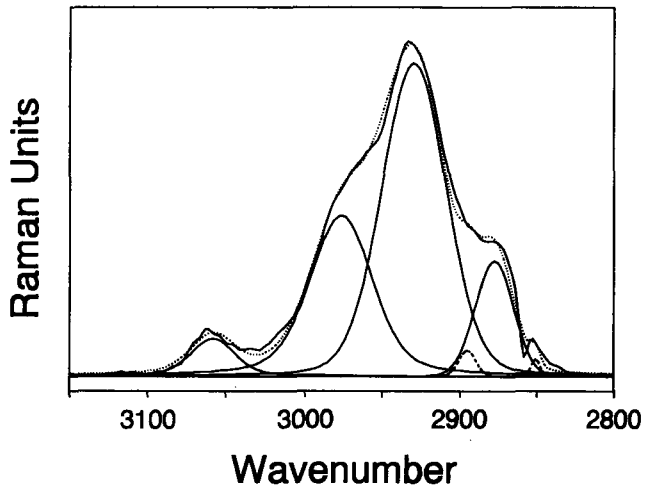

FIG. 3. The deconvolution of the 2800 to 3150 cm^{-1} region of the Fourier transform-Raman spectrum obtained from scoured Merino wool. The raw data (—), six component peaks (wool wax - - - -, wool —) and their composite (• • • • •) are depicted.

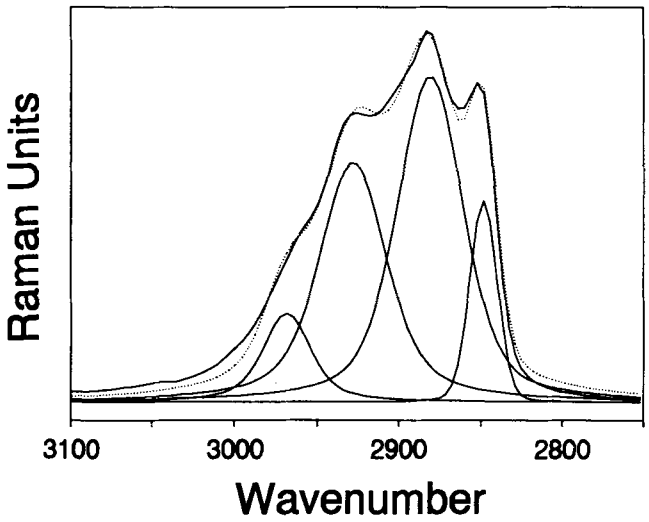

FIG. 4. The deconvolution of the 2750 to 3100 cm^{-1} region of the Fourier transform-Raman spectrum obtained from wool wax. The raw data (—), four component peaks (—) and their composite (· · · ·) are depicted.

parameters and vibrational assignments of the component bands, obtained for both the scoured wool and wool wax spectra, are given in Table 1. The vibrational assignments are based on those reported for model compounds (15–17).

Component band analysis of the wool wax shows four peaks as expected, while that of the scoured wool gave a minimum of six peaks. The deconvoluted scoured wool spectrum exhibits a weak component at 2895 cm^{-1}, which lies between the two major wax peaks (2880 and 2928 cm^{-1}) and is attributed to residual wax and structural lipid material. The fact that this band grows with increasing wax content in the raw wool confirms this assignment. The intensity of the band at 2850 cm^{-1} also increased with increasing wax content because it is actually a composite of the CH_2 symmetric stretching vibrations of the wool, the wool wax and the lipid material. The component of this band arising from the wool wax becomes more and more dominant as the wax content of the sample increases. The C-N-H bending overtone and three other C-H stretching vibrations, originating from the wool fiber located at 2877, 2930 and 2976 cm^{-1}, complete the set of six component bands used for band fitting the spectrum obtained from the scoured wool sample.

Initially, all eight of the bands assigned to C-H stretching modes (four each from the wax and wool), shown in Table 1, were used to fit the raw wool spectra. The quantitative results obtained were not consistent when checked against those of the solvent extraction method. Moreover, band fitting based on these eight component bands did not produce a unique solution. Band deconvolution work on other chemical systems suggest that the most meaningful band fit is generally achieved when the number of bands necessary to obtain the "best" fit is kept to a minimum (18). Six components, four representing the wool, as given in Table 1, and two from the wax, centered at 2849 and 2895 cm^{-1}, were found to suffice with respect to the above requirements. The reduction in the number of components representing the wool wax is achieved by combining the strong bands at 2889 and 2928 cm^{-1} into a single broader component at 2895 cm^{-1} and by ignoring the 2966 cm^{-1} component due to its extremely weak intensity. For this wax quantitation work, we have also introduced further constraints to enable optimum band fitting and to ensure that component bands obtained reflect the band characteristics seen in the scoured wool and wool wax band profiles. For instance, the centers and relative widths of the overlapping bands at 2850, 2877, 2895 and 2930 cm^{-1} were constrained during band fitting to match those found for the isolated scoured wool and wool wax components. The results of a band fitting exercise for a spectrum obtained from a sample of raw wool containing approximately 30% wax are shown as in Figure 5.

The percentage of wax in a raw wool sample is obtained by taking the ratio of the sum of the areas of the wool wax component peaks to the sum of the areas of all five of the C-H stretching component peaks of the raw wool band. The C-N-H bending overtone is used in band fitting but omitted from the wax content calculations. If the residual wax content of the scoured wool sample is calculated by this method, a value of 1.8% wax is obtained. The solvent-extractable content of this sample, which includes residual wax and scour detergent as well as other fatty matter, was determined to be 0.5%. This value is typical of that found for scoured wool. The 1.3% difference observed between these two methods can be attributed to nonextractable fatty matter, such as wool lipids, as well

TABLE 1

Band Fit Parameters and Vibrational Assignments for the Component Peaks of Scoured Merino Wool (Refs. 15,16) and Wool Wax (Ref. 17) in the 2700 to 3200 cm^{-1} Region

Sample	Peak center (cm^{-1})	Peak width at half-height (cm^{-1})	Peak amplitude (Abs)	Peak assignment
Scoured wool	2850	8	0.02	Symmetric CH_2, residual wax and lipid matter
	2877	31	0.10	Symmetric CH_3
	2895	15	0.03	Residual wax and lipid matter
	2930	58	0.27	Asymmetric CH_2
	2976	50	0.13	C^{α}-H and Asymmetric CH_3
	3057	31	0.03	2ν C-N-H bend
Wool wax	2849	23	0.56	Symmetric CH_2
	2880	54	0.91	Symmetric CH_3
	2928	54	0.67	Asymmetric CH_2
	2966	39	0.25	Asymmetric CH_3

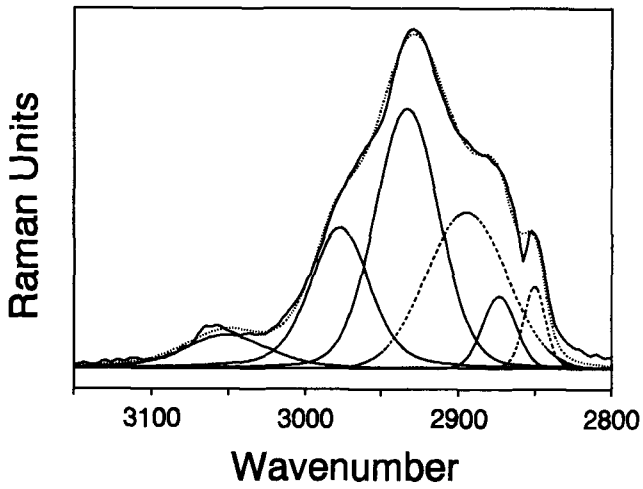

FIG. 5. The deconvolution of the 2800 to 3150 cm^{-1} region of the Fourier transform-Raman spectrum obtained from raw Merino wool containing approximately 30% wax. The raw data (—), six component peaks (wool wax - - -, wool —) and their composite (· · · ·) are depicted.

as to experimental error. The extraction method does not determine the total wax and lipid content, only the solvent-soluble portions of these components. Different solvent extraction systems remove different amounts of these materials (14). The Raman technique, being a bulk technique on the other hand, would detect the wool wax as well as all other fatty matter present.

Table 2 compares the results obtained from the Raman quantitation technique with those of the solvent extraction method for samples of raw Merino wool containing varying amounts of wax. As raw wool samples with wax contents less than 15% were not available, samples of approximately 10, 5 and 3% wax were prepared. The results of the analysis of these laboratory-prepared "raw wool" samples are shown in Table 3. As can be seen by comparing the "Average wax" values determined by the two techniques, good agreement was found. In general, the precision of the Raman spectroscopic method was better than that obtained from the extraction method.

As mentioned earlier, the Raman spectral method would yield wax content values that are approximately 1% higher than that of the extraction method due to the detection of nonextractable lipid matter. Therefore, it follows that the wax values determined by the Raman method should be corrected for the inherent difference between the two techniques by subtraction of a lipid component of 1.3% as estimated from the scoured wool samples. From comparison of the "Corrected wax" values given in Tables 2 and 3 to the results obtained by extraction, a significant improvement between the correlation of the two methods is observed for all samples.

A possible source of error in the Raman method arises from the fact that the 2850 cm^{-1} band is attributed solely to the wax component. This treatment of the data will induce negligible error for samples with high wax content, but at low concentrations this may not be the case. If the residual wax content of the scoured wool was recalculated assuming that the 2850 cm^{-1} band is due only to the wool, a wax content value of 1.4% is obtained. This decrease of 0.4% does not have any significant effect on the correlation between the two methods.

The degradation of wool wax by weathering is believed to involve the hydrolysis of ester linkages and oxidation of the steroid groups. Considering the vibrational modes used in the Raman spectral method, neither of these chemical changes would be expected to have significant effects on the wax content determination. The effects of common raw wool contaminants, such as vegetable matter (VM) and polyethylene (PE), on the results of the analysis were also investigated. Because the laser beam impinges upon only a small portion of the raw wool sample, any interference with the beam by foreign matter would be expected to have great consequences. For example, VM scatters light poorly, and its presence, even in small amounts, would result in an extremely weak spectrum. On the other hand, polymeric materials such as PE scatter light strongly, and their presence would significantly increase the intensities of the C-H vibrational modes used in the analysis. Because all spectral measurements in this study used a small sample mass, the presence of such macroscopic contaminants would unlikely escape visual detection. In any case, large signal amplitude differences would be observed between the three runs of the same sample if the laser beam hit any VM or polymeric material. We did not observe any such differences in our study.

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TABLE 2

Comparison of Solvent Extraction and Raman Spectral Methods for the Determination of Wax in Samples of Raw and Scoured Merino Wool

Sheep type	Fleece position	Quantitation method	Average wax (%)	Standard deviation	Corrected[a] wax (%)
Merino 1	Back	Extraction	31.5	0.8	31.5
		Spectral	33.4	0.6	32.1
Merino 1	Middle	Extraction	27.2	1.6	27.2
		Spectral	29.3	0.3	28.0
Merino 2	Back	Extraction	18.2	1.3	18.2
		Spectral	20.1	0.3	18.8
Merino 2	Front	Extraction	14.8	1.1	14.8
		Spectral	16.3	0.4	15.0
Scoured Merino	Bulk	Extraction	0.5	0.06	0.5
		Spectral	1.8	0.3	0.5

[a]No correction applied to extraction-based values.

TABLE 3

Comparison of Extraction and Spectral Methods for the Laboratory-Prepared Greasy Wool Samples

Quantitation method	Average wax (%)	Standard deviation	Corrected[a] wax (%)
Extraction	9.9	1.2	9.9
Spectral	10.9	0.1	9.6
Extraction	5.8	0.6	5.8
Spectral	6.9	0.4	5.6
Extraction	3.0	0.5	3.0
Spectral	4.1	0.6	2.8

[a]No correction applied to extraction-based values.

time taken for each wax determination cycle was approximately 80 min. This time was reduced to 20 min by collecting the spectra while using the optimized parameters and computer-averaging the three spectra prior to component band analysis and wax content determination. The results obtained with the throughput optimized method were almost identical in accuracy and precision to those obtained by the 80-min analysis cycle.

This study has demonstrated that the quantitation of wool wax in raw wool is achievable by a Raman spectroscopic component band analysis method. The results of Raman and extraction-based measurements, performed on samples spanning the range between 32 and 0.5% (w/w) wax in wool, were in excellent agreement, once they were corrected for the nonextractable fatty matter content of the wool. The fact that this correction is necessary constrains the lower limit of wax determinable to near 3%, making this method untenable for low wax concentrations such as the residual wax content of scoured wool.

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REFERENCES

1. Motiuk, K., *J. Am. Oil Chem. Soc. 56*:91 (1979).
2. Motiuk, K., *Ibid. 56*:651 (1979).
3. Motiuk, K., *Ibid. 57*:145 (1980).
4. Australian Standard Method 2001.3.4, Standards Australia, North Sydney 1981.
5. International Wool Testing Organisation Method 10-62(E), The International Wool Secretariat, London 1988.
6. Hammersley, M.J., S.L. Ranford and V.C. Acker, IWTO Report No. 18, International Wool Textile Organisation, Brussels, June 1988.
7. Connell, J.P., and K.H. Norris, *Textile Res. J. 50*:371 (1980).
8. O'Keefe, J.H., and L.F. Sharry, *J.A.O.A.C. 65*:172 (1982).
9. Carter, E.A., P.M. Fredericks, J.S. Church and R.J. Denning, *Spectrochim. Acta*, in press.
10. Church, J.S., A.S. Davie, D.W. James, W.H. Leong and D.J. Tucker, *Appl. Spectrosc.48*:813 (1994).
11. Faiman, R., and D.A. Long, *J. Raman Spectrosc. 3*:371 (1975).
12. Faiman, R., and D.A. Long, *Ibid. 3*:379 (1975).
13. Larson, K., and R.P. Rand, *Biochim. Biophys. Acta 326*:245 (1973).
14. Rivett, D.E., *Wool Sci. Rev. 67*:1 (1991).
15. Diem, M., P.L. Polavarapu, M. Oboodi and L.A. Nafie, *J. Am. Chem. Soc. 104*:3329 (1982).
16. Frushour, B.G., P.C. Painter and J.L. Koenig, *J. Macromol. Sci.-Rev. Macromol. Chem. C15*:29 (1976).
17. Bulkin, B.J., and K. Krishnan, *J. Am. Chem. Soc. 93*:5998 (1971).
18. Shurvell, H.F., and J.T. Bulmer, in *Vibrational Spectra and Structure*, Vol. 6, edited by J.R. Durig, Elsevier Scientific, New York, 1977, pp. 91-172.

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