A Rapid Test for the Identification of Incense Resins

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Olibanum, or frankincense, is a gum resin that has been collected since ancient times from trees of the genus *Boswellia*. There are about a dozen species of *Boswellia*, which grow in India, Arabia, and the eastern coast of Africa,[1] of which four have been important commercial sources of olibanum.[2]

Olibanum has been used as an incense since Biblical times and is still used in Roman Catholic, Episcopal, and Eastern Orthodox churches. It also has an important use as a fixative in perfumes, soaps, creams, lotions, and detergents.[3]

The olibanum that is used commercially in the United States in the perfume industry and in incense manufacture comes from three countries, India, Eritrea, and Somalia, and that used in Europe comes also from Aden. These resins differ in their properties and it is expected that they differ also in their botanical origin (species). Consequently, methods for distinguishing them and for identifying their respective species would seem to be essential.

Several studies to determine the essential oils and acidic and gum components of olibanum have been reported.[4-9] These studies used such methods as gas chromatography and mass spectrometry for the determinations. However, in many studies no identification of the origin of the olibanum used has been given, a fact which has severely limited their value to research. Two German studies do address the chemical differences between the olibanum obtained from Aden and Eritrea.[8,9] However, there should be no reason to assume that one particular species only grows in one particular region.

The failure to identify samples by source has produced great confusion in our understanding of the nature of the olibanum resins sold in Europe and the United States. When a worker obtains data that conflict with previously reported findings, interpretation becomes difficult.[8] Furthermore, uncertainty about the biological identity of any of the varieties of olibanum in commercial use has led to great confusion regarding their very real differences and has sometimes made it difficult to obtain the best material for the particular application desired.

This difficulty is especially acute in the church incense manufacturing industry. The olibanum currently sold as "Indian olibanum" gives an odor resembling turpentine or burning rubber when it is "burned" (pyrolyzed) on charcoal. Church incense manufacturers, ignorant of this difference between "Indian olibanum" and the other types of olibanum have switched their incense base to "Indian olibanum" when that variety is more readily available or cheaper. Consumers are dissatisfied, but ignorance of the cause of their dissatisfaction makes it impossible for them to lodge a complaint that will demand attention.

It was our dissatisfaction, as consumers, with most of the available church incense that aroused our interest in this project. Perfumers, we discovered, must depend on non-specific tests such as color, odor, and acid number or on elaborate expensive procedures such as gas chromatography. We were not satisfied to depend on color and odor alone and we did not have the time or the instrumentation for acid number determinations or gas chromatography. We felt that a rapid test to distinguish the varieties of commercial olibanum was essential. We have developed a rapid and simple test that uses only materials that are readily available from any chemical supplier.

We have found this test to provide positive identification of the varieties of olibanum used by the perfume and church incense industries in the United States. It has proved very helpful to us

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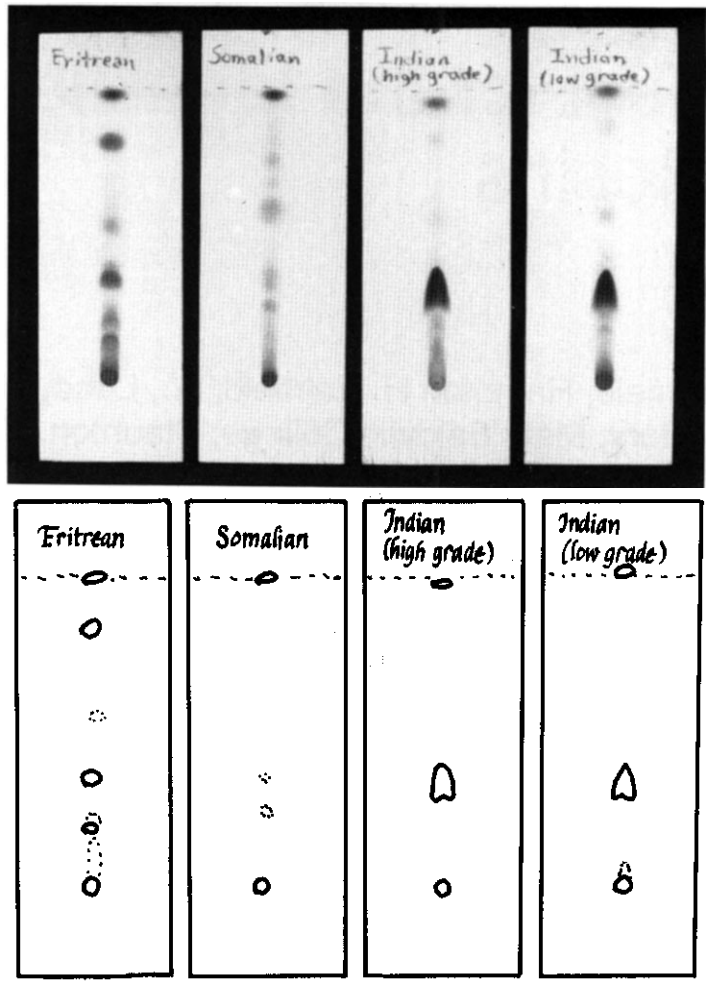

Figure 1

in screening samples of olibanum that we were considering purchasing. Furthermore, we feel that this test will allow us to identify the species of olibanum that are now available commercially. We also hope our test can be used to follow changes in botanical origin.

We report here the detailed procedure for conducting the test and for interpreting the results.

Experimental

A sample of olibanum (approx. 0.2 g) is extracted with 1 ml of ethyl alcohol, (reagent grade solvent alcohol, absolute alcohol, or pure grain alcohol, 190 proof, may be used). The simplest procedure for the extraction is to cover the olibanum tears with alcohol in a test tube stoppered with a cork and to shake the mixture occasionally at room temperture over a period of approximately thirty minutes.

One or two μl of the extract is removed into a capillary and spotted onto a thin-layer chromatography slide coated with silica gel. (Baker-flex 2.5cm x 7.5cm flexible sheets coated with silica gel 1B2-F [cat. no. 4449] pre-dried 1 hour at 110°C give excellent results.) The spots are allowed to dry and the slides are developed with methylene chloride or chloroform and visualized with iodine vapor or with ceric sulfate reagent.

The chromatogram is visualized with iodine vapor by placing a few crystals of iodine in a covered jar and inserting the slide for five to ten minutes. The vapor dissolves in the spot of organic material to produce a brown color, which fades upon exposure to air.

The chromatogram is visualized with ceric sulfate reagent by dipping it into a solution of ceric ammonium sulfate in sulfuric acid prepared as follows: 12 g ceric ammonium sulfate are dissolved in 100 ml of 1.75% sulfuric acid. After the slide is dipped into this solution it is heated on a hot plate at a medium setting. The ceric sulfate reacts with the terpenoid components to produce lavendar to purple spots. (The hot plate setting chosen is the highest one possible that does not melt the plastic backs of the slides—350-400 on a Thermolyne Model HP-A1915B hot plate works well.)

Results and Discussion

The test has been run on twelve samples of incense from six manufacturers and on samples of olibanum from three wholesale and two retail suppliers as well as on several samples of known botanical source supplied to us by the Royal Botanic Gardens at Kew, Richmond, England. Each sample was independently identified by odor when burned on charcoal. The thin-layer chromatography test was shown to be totally reliable. The chromatograms produced by Ethiopian, Somalian, and Indian are shown in Figures 1 and 2.

In Figure 1 are shown the chromatograms visualized with iodine vapor. In addition to the (brown) spot at each origin, the Eritrean (Ethiopian) olibanum produces distinct spots at Rf values of 1.0, 0.83, 0.36, and 0.20; the Somalian olibanum produces a distinct spot at an Rf value of 1.0, and the Indian olibanum produces a distinct shield-shaped spot at an Rf value of 0.36. (This shield-shaped spot came up green at first and then changed to brown.)

In Figure 2 are shown the chromatograms visualized with ceric sulfate reagent. In addition to a band (of purple) near each origin, the Eritrean (Ethiopian) olibanum is characterized by spots at Rf values of 1.0 (faint), 0.83, 0.36, and 0.20; the Somalian olibanum is characterized by spots at Rf values of 1.0, 0.76, 0.60 (very strong), and 0.36 (faint); and the Indian olibanum is characterized by a shield at an Rf value of 0.36.

It seems especially significant that, although all three varieties of olibanum produce a spot at an Rf value of 0.36, each variety produces a spot that has a characteristic intensity and shape. Furthermore, the appearance with ceric sulfate reagent of a spot at Rf 0.83 with no spot around Rf 0.76, identifies a sample as Ethiopian olibanum; the appearance with ceric sulfate reagent of a spot at Rf 0.76 identifies a sample as Somalian olibanum; the absence with ceric sulfate reagent of any spot at an Rf value greater than 0.40 identifies a sample as Indian olibanum.

Evidence for the botanical identity of the resins is shown in Figures 3 and 4. In Figure 3 are

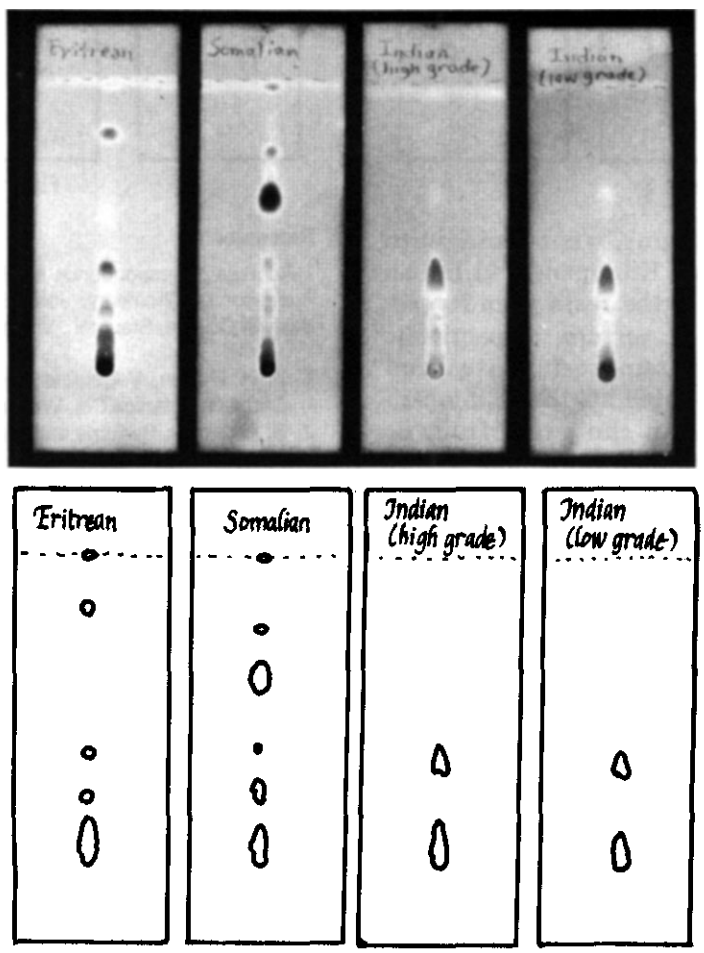

Figure 2

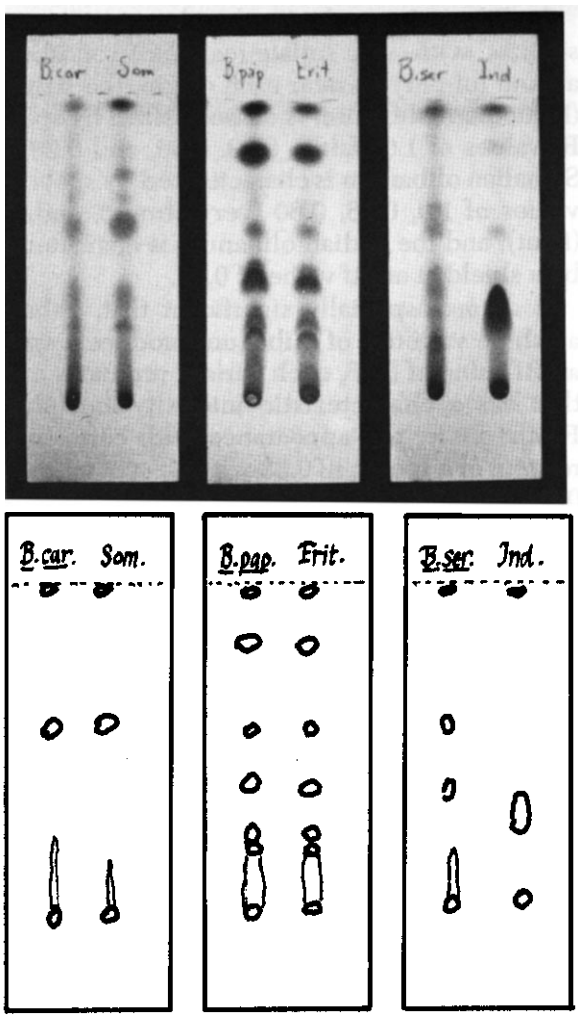

Figure 3

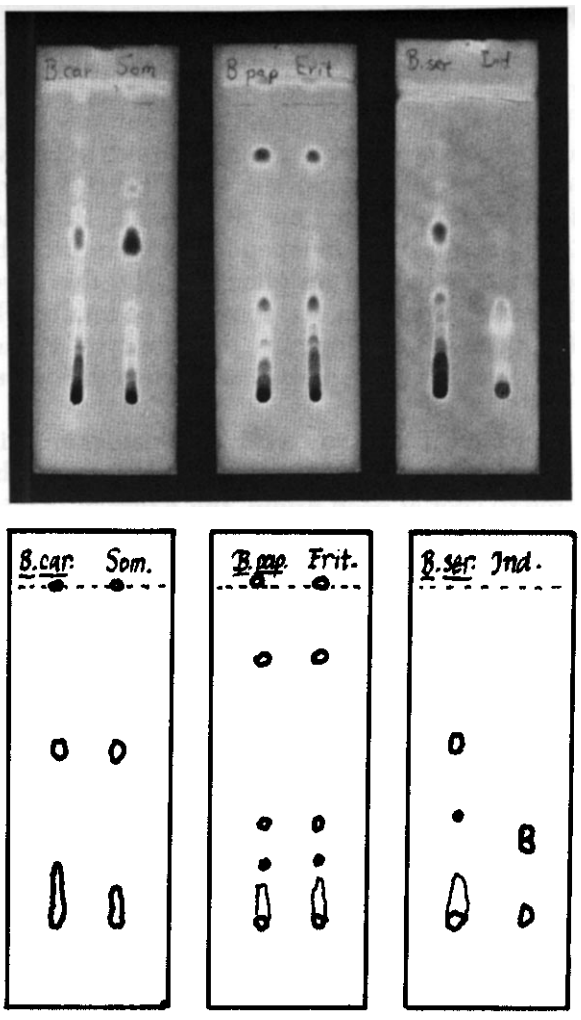

Figure 4

shown the chromatograms, visualized with iodine vapor of Somalian, Ethiopian, and Indian olibanum compared with the resin from *B. carteri, B. papyrifera,* and *B. serrata,* respectively. In Figure 4 are shown the same resins, visualized with ceric sulfate. It is obvious that Ethiopian olibanum is identical to the resin from *B. papyrifera* and that Somalian olibanum is very similar to the resin from *B. carteri.*

We are continuing our investigation into identifying the botanical source of the Indian olibanum. We are also investigating the identity of the compounds responsible for certain of the characteristic spots produced by the resins.

Acknowledgments

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