to 5. The IR and PMR spectra are in accordance with the structure 5.

A mixture of 1 (0.5 g, 0.0012 mol) and borontrifluoride etherate (3 ml) in dry dioxan (15 ml) was stirred at room temperature for 15 h under $N_2$ atmosphere. Excess borontrifluoride etherate was removed under reduced pressure. After adding ice-cold water (30 ml) to the reaction mixture, the precipitated solid was extracted with diethyl ether (3 × 30 ml), ether extract washed with water (3 × 20 ml) and dried ($Na_2SO_4$). After evaporating the ether, the solid residue was column chromatographed (40 × 1 cm) using chloroform as the eluant. TLC monitored first fraction was separated, condensed to 5 ml and the petroleum ether (40–60°) was added when 5 was obtained as a white solid.

Yield 0.42 g (88%), m.p. 212–14° (lit. 214–15°)[7]. IR (nujol): 1760 (C=O), 1700 (C=C), 1600 $cm^{-1}$ (aromatic C=C), PMR ($CDCl_3$): $\delta$ 3.75 (s, 11H, ($OCH_3$) and $C_9$-H), 4.8(*bs*, 3H, $C_1$-H and $C_4$-H), 5.90 (*s*, 2H, O-$CH_2$-O), 6.30 (*s*, 2H, $C_2$,-H and $C_6$,-H) 6.60 (*s*, 1H, $C_5$-H), 6.70 (*s*, 1H, $C_8$-H).

28 March 1988


1. Wilson, L., *Ann. N. Y. Acad. Sci*, 1975, **253**, 213; Wilson, L., Bamberg, J. R., Mizel, S. B., Grisham, L. M. and Creewell, K. M., *Fed. Proc. Fedn. Am. Soc. Exp. Biol.*, 1974, **33**, 158.
2. Smissman, E. E., Murray, R. J., McChesney, J. D., Houston, L. L. and Pazdernik, T. L., *J. Med. Chem.*, 1976, **19**, 148; Jungi, W. F. and Senn, H. J., *Cancer Chemother. Rep.*, 1975, **59**, 737; Rivera, G., Avery, T. and Pratt, C., *Cancer Chemother. Rep.*, 1975, **59**, 743; Keller-Juslin, C, Kuhn, M. and Von Wartburg, A., *J. Med. Chem.*, 1971, **14**, 936.
3. Hartwell, J. L. and Schrecker, A. W., *Fortschr. Chem. Org. Naturst.*, 1958, **15**, 83.
4. Gensler, W. J. and Gatsonis, C. D., *J. Org. Chem.*, 1966, **31**, 3224.
5. Schreier, E. A., *152nd National Meeting of the American Chemical Society*, New York, Sept. 1976, Abstracts Sect. P.No. 34, Dr Schreier very kindly made the full text of his lecture available to us.
6. Schrecker, A. W. and Hartwell, J. L., *J. Am. Chem. Soc.*, 1952, **74**, 5676.
7. Anjanamurthy, C. and Lokanatha Rai, K. M., *Indian J. Chem.*, 1982, **B21**, 62.


## HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF CERTAIN PHENOLS AND PHENOLIC ACIDS FROM PLANTS

T. K. ANNAPURANI
*Public Health Laboratory, Indiranagar, Pondicherry 605 006, India.*

HIGH performance liquid chromatography[1–3] (HPLC) is one of the most efficient among the recent analytical techniques of plant analysis. It is superior to other similar techniques like TLC, GLC, etc. in terms of the universality of application and reproducibility of results. A recent modification[4], on line HPLC, combining the recording of UV-visible absorption spectra of individual eluate in MeOH with the presence of certain diagnostic reagents has revolutionized the easy identification of plant phenolics. The identification of phytotoxins and phytoalexins in plants under diseased conditions is an important aspect of phytochemical applications in plant pathology for which HPLC analysis is of great use. During our current work[5] on the abnormal constituents of *Sorghum vulgare* affected by different fungi, we observed the presence of certain phenolic compounds responsible for offering resistance to microbial attack. To facilitate easy identification of these unusual phenolic compounds by HPLC it was necessary to keep the HPLC pattern for common plant phenols under standardized conditions. The HPLC data acquired for twenty phenolic or acidic phytochemicals using Shimadzu LC-6A liquid chromatograph are reported in this note. The test samples were either isolated from plant sources[6] or procured from commercial firms.

Solutions were prepared in methanol and a concentration of 0.5–1 mg per ml was used. Both Zorbax C8 and Zorbax ODS (C18) reverse phase columns (4.6 mm i.d. × 25 cm) with 10% acetic acid:methanol (6:4) as mobile phase using a constant flow rate of 1 ml/min were employed. Pressures of 1 × 100 kg F $cm^{-2}$ and 1.8 × 100 kg F $cm^{-2}$ respectively and an ambient temperature were maintained. UV absorption was done at 280 nm. Concentrations were determined by area integration with an automatic integrator. The results are presented in tables 1 and 2. The lower detection limit was around 0.1 $\mu$g.

The results indicate that HPLC analysis can profitably be used for qualitative and quantitative analysis of common plant phenols and acids. The

**Table 1** *Retention time of different plant phenolic acids*

| Compound | ODS (min) | C8 (min) |
|---|---|---|
| Gallic acid | 2.7 | 3.1 |
| Methyl gallate | 3.4 | 3.7 |
| 3,4 di-OH cinnamic acid | 3.8 | 4.0 |
| Ethyl gallate | 4.4 | 4.5 |
| 4-OH cinnamic acid | 5.1 | 5.1 |
| Benzoic acid | 7.6 | 6.4 |
| Salicylic acid | 9.2 | 7.1 |
| 3,4, di-OH benzoic acid | 3.1 | 3.4 |
| 4-OH-3-OMe benzoic acid | 3.9 | 4.2 |
| Ferulic acid | 5.1 | 5.2 |
| 3,4 di-OMe benzoic acid | 5.4 | 5.6 |
| 4-OMe benzoic acid | 8.3 | 7.1 |
| Iso ferulic acid | 11.4 | 7.5 |
| Cinnamic acid | 12.9 | 9.2 |

**Table 2** *Retention time of different plant phenols*

| Compound | ODS (min) | C8 (min) |
|---|---|---|
| Quinol | 3.0 | 3.3 |
| Resorcinol | 3.3 | 3.5 |
| Catechol | 3.9 | 3.7 |
| Orcinol | 4.0 | 3.8 |
| Vanillin | 5.0 | 4.5 |
| Phenol | 5.8 | 4.6 |
| Cresol | 9.4 | 5.8 |

components could be easily separated and identified from inseparable mixture in PC and TLC (the components listed in tables 1 and 2, were separated from each other in the group.) A PC homogeneous phenolic compound showing a marked anti-cancer activity when subjected to HPLC revealed it to be a mixture of three components (protocatechuic, ferulic and caffeic acids in the ratio of approximately 3:2:2). Zorbax ODS was found superior to Zorbax C8 in terms of resolution. HPLC analysis of phenolic components of non-resistant and resistant strains of *S. vulgare* is now in progress.

Thanks are due to Dr A. G. Ramachandran Nair, Department of Chemistry, Pondicherry University for encouragement, guidance and supply of several samples.

25 April 1988

1. Kingston, D. G. I., *J. Nat. Prod.*, 1979, **42**, 237.
2. Linskens, H. F. and Jackson, J. F., *High performance liquid chromatography in plant sciences*, Springer-Verlag, London, 1987.
3. Decosterd, L. A., Dorsaz, A. C. and Hostettmann, K., *J. Chromatogr.*, 1987, **406**, 367.
4. Hostettmann, K., Domon, B., Schaufflberger, D. and Hostettmann, M., *J. Chromatogr.*, 1984, **283**, 137.
5. Annapurani, T. K., Ramaraj, B. and Nair, A. G. R., Unpublished work.
6. Majority of the samples were collected from the stock of Dr A. G. R. Nair, Pondicherry University.

## INTERACTION OF TRIS (2-ETHYL HEXYL) TRIMELLITATE (HATCOL-200) COTTON SEED OIL AND NORMAL SALINE V ITH PENTOBARBITAL SODIUM—A PROSPECTIVE APPROACH

K. RATHINAM and P. V. VEDANARAYANAN
*BMT Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum 695 012, India.*

THE application of tris (2-ethyl hexyl) trimellitate (HATCOL-200) as a primary plasticizer in polyvinyl chloride used for medical applications (such as plastic tubes, blood bags, dialysis tubing, catheters and similar biomedical devices) has long been in practice. Blood serum extraction studies of film made with HATCOL-200 showed extractables to be one hundredth of that of a film made with di (2-ethyl hexyl) phthalate (DEHP), the conventionally used plasticizer[1]. Since the new plasticizer is considered as an alternative for and structurally analogous to DEHP (figure 1), its effect on biological systems is explored here by studying its interaction with other xenobiotics, such as pentobarbital. Sleeping time (narcosis) index of pentobarbital administered was used to investigate its interaction with HATCOL-200, cotton seed oil and normal saline with a view of obtaining meaningful data towards their mechanism of action.

Adult male albino mice (17–23 g) from the stock at this Institute and maintained on pellet diet and water *ad libitum* under standard husbandary conditions were used. These animals were divided into three groups of six each. They were treated with HATCOL-200, cotton seed oil and normal saline (0.9 g w/v in distilled $H_2O$) [5 ml/kg] for five days intraperitoneally. Doses were selected on the basis of our pilot studies.