(12) Schnider, O. S., and Grüssner, A., *Helv. Chim. Acta*, **32**, 821(1949); through *Chem. Abstr.*, **43**, 6635(1949).
(13) Van Campen, M. G., Meisner, D. F., and Parmerter, S. M., *J. Am. Chem. Soc.*, **70**, 2296(1948).
(14) Leonard, N. J., and Leubner, G. W., *ibid.*, **71**, 3408 (1949).
(15) Shelton, R. S., Van Campen, M. G., Jr., Meisner, D. F., Parmerter, S. M., Andrews, E. R., Allen, R. E., and Wyckoff, K. K., *ibid.*, **75**, 5391(1953).
(16) Kershaw, J. R., and Uff, B. C., *Chem. Commun.*, **1966**, 331.
(17) Weinstock, J., and Boekelheide, V., "Organic Synthesis," coll. vol. IV, Rabjohn, N., ed., John Wiley & Sons, Inc., New York, N. Y., 1963, p. 641.
(18) Boekelheide, V., and Weinstock, J., *J. Am. Chem. Soc.*, **74**, 660(1952).
(19) Craig, P. N., Nabenhauer, F. P., Williams, P. M., Macko, E., and Toner, J., *ibid.*, **74**, 1316(1952).
(20) Gibson, H. W., Popp, F. D., and Noble, A. C., *J. Heterocyclic Chem.*, **3**, 99(1966).

Plasticizers in Medical Application I

Analysis and Toxicity Evaluation of Dialkyl Benzenedicarboxylates

By JAY NEMATOLLAHI, WALLACE L. GUESS, and JOHN AUTIAN

A series of dialkyl and dicycloalkyl benzenedicarboxylates (phthalates, isophthalates, terephthalates), of which some have been employed as plasticizers, were synthesized and purified. Thin-layer chromatography and infrared spectrometry were employed as methods of detection and identification of these compounds. Toxicity tests were conducted using mammalian cell cultures and limited mouse studies. The low molecular weight esters and their isomers manifested toxic responses while the esters containing alkyl groups C_6 to C_9 were devoid of toxic effect. The di-*n*-decyl phthalate and terephthalate also elicite toxic actions to cells. Results from this study indicate the need for good quality control procedures to prevent the access of potentially toxic plasticizers in devices for medical and paramedical applications.

A NUMBER of polymeric materials require the presence of one or more plasticizers to impart a degree of softness and flexibility not inherent in the parent polymer. The concentration of the plasticizer may vary from a rather small percentage to as high as 60%, depending upon the desired final properties. Selection of a plasticizer (or plasticizers) for a specific plastic is dependent upon a number of considerations; the initial consideration is that it will impart to the final plastic the optimum properties desired at a minimum cost consistent with quality production. If the plastic material is to be used as a food wrap or a container, the degree of migration of the plasticizer to the food and the toxicity profile of the migrated ingredient must be evaluated under the Food Additive Amendment. Development and use of the plasticized polymeric materials for various medical, pharmaceutical, and dental applications such as tubing, catheters, containers, and protective films raises the question as to the toxic potential of migrated plasticizer which may enter the patient by routes other than oral. In this regard, consideration must also be given to the exact composition of the "migrated ingredient" since it may actually be composed of the intact plasticizer, degraded products of the plasticizer, impurities in the plasticizer, and other additives such as stabilizing agents, antioxidants, degraded products of the additives, and impurities in the additives.

Within the past 5 years, a number of published reports (1–3) have demonstrated that certain types of polyvinyl chloride (PVC) devices could produce toxic effects if one or more ingredients migrated from the device into tissue or into an injectable solution.

Recent investigations (4) on a group of PVC blood bags revealed that certain ingredients were migrating from the plastic into acid citrate dextrose solutions. The combined ingredients were found to be toxic to mammalian cell cultures. Further investigations on the "leached" ingredients showed the presence of several chemicals, presumably plasticizers together with their degradation products. A frequently employed plasticizer in the PVC plastic used for the manufacture of the blood bags was found to be di(2-ethylhexyl)phthalate. Others such as dialkyl sebacates and epoxidized soybean oil were also detected in the PVC.

When a combined chemical and spectrophotometric method was employed for the detection and identification of these additives, it became obvious that a mixture of homologs of a few di-

Received May 18, 1967, from the Drug-Plastic Research and Toxicology Laboratories, College of Pharmacy, University of Texas, Austin, TX 78712
Accepted for publication July 12, 1967.
This investigation was supported in part by grant CA 06120-06 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
The authors thank Mrs. Nawal Shafiq for her technical assistance.

alkyl phthalates could not be separated easily and under these experimental conditions their infrared spectra were indistinguishable. Likewise, when a commercial medical grade di(2-ethylhexyl) phthalate (DOP) was examined by thin-layer chromatography (TLC), the presence of several spots was observed. Again it was not possible by tedious column chromatography to separate some of the proximately located spots observed on the thin-layer plate, and the I.R. spectra of the fractions did not provide sufficient help for structural elucidation. This, and the fact that the commercial synthesis of plasticizing esters does not rule out the presence of isophthalates and terephthalates in phthalate plasticizers, necessitated the extension of this investigation to the synthesis and analysis of a series of authentic esters of the three isomers of benzenedicarboxylic acids. The detected TLC spots for the medical grade DOP, mentioned above, could be ascribed to the homologs of the phthalic acid esters as well as to its positional isomers (see Table I). One of the industrial methods of preparation of benzenedicarboxylic acids is *via* oxidation of xylenes. Quite often one isomer of xylene contains the other two, whose oxidation would give rise to a mixture of the three corresponding acids.

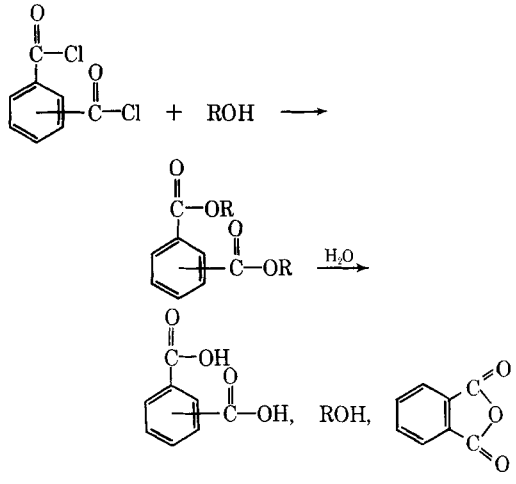
TABLE I—R_f VALUES FOR DIALKYL BENZENEDICARBOXYLIC ACIDS. ADSORBENT, SILICA GEL, DEVELOPER, ETHYL ACETATE–*n*-HEXANE (10:90)

R	Phthalate	Iso-phthalate	Tere-phthalate
CH_3	.27	.41	.56
C_2H_5	.42	.54	.79
n-C_3H_7	.55	.66	.82
iso-C_3H_7	.59	.67	.82
n-C_4H_9	.72	.76	.93
iso-C_4H_9	.76	.77	.92
n-C_5H_{11}	.83	.84	.95
iso-C_5H_{11}	.85	.86	.94
Cyclo-C_5H_9	.73	.77	.85
n-C_6H_{13}	.89	.86	.96
Cyclo-C_6H_{11}	.81	.81	.88
n-C_7H_{15}	.90	.92	.97
Cyclo-C_7H_{13}	.79	.92	.95
n-C_8H_{17}	.90	.93	.98
Cyclo-C_8H_{15}	.83	.85	.97
n-C_9H_{19}	.93	.94	.98
n-$C_{10}H_{21}$	.93	.95	.99
n-$C_{11}H_{23}$	.94	.96	.99
n-$C_{12}H_{25}$	.95	.97	.99

The main objectives of this investigation were: (*a*) to synthesize a homologous series of the authentic dialkyl and dicycloalkyl benzenedicarboxylates in order to acquire information on certain physical properties and spectroscopic data for the series, requisite both for present and further investigations in plastics; (*b*) to search for the existence of a correlation among chemical structures of the esters and their I.R. spectral patterns; (*c*) to correlate the chemical structures of the esters together with their products of hydrolysis to their toxic potential on the mammalian cell cultures and mice; and (*d*) to determine the rate of hydrolysis of the esters and relate this rate to chemical structure and toxic effect. The latter part will be discussed in another paper.

CHEMISTRY

The rapid rate of reaction of phthalyl, isophthalyl, and terephthalyl chloride with alcohol suggested the adoption of this route for the preparation of dialkyl and dicycloalkyl benzenedicarboxylates. The acid chlorides were employed slightly in excess of the stoichiometric quantity of the alcohol in order to facilitate the purification of the final product which consists of the removal of the acid from the ester by ether–dilute aqueous base extraction. In addition to *n*-alkyl and some iso-alkyl esters, four cycloalkyl esters were prepared: cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl benzenedicarboxylate. The general method of synthesis and the degradation by hydrolysis of the esters is shown in Scheme I.



Scheme I

Due to cell culture toxicity manifested by some esters and the products of hydrolysis of the esters of benzenedicarboxylic acids, the establishment of a detection method for esters mixed with such products became essential. This purpose was served well by the application of thin-layer chromatography. The esters, acids, and anhydride (the anhydride formed as one of the products of degradation of phthalic acid esters) could be detected visually on the thin-layer plate by the aid of a short wave ultraviolet light. Both the acid and anhydride remained near the origin whereas the esters showed a noticeable R_f value. The position of the alcohol on the TLC plate usually was in between the ester and acid. Since the presence of the alcohol could not be visualized by the aid of a U.V. light, its location was fixed by spraying the plate with a 10% solution of phosphomolybdic acid in 95% ethanol.

TABLE II—RESULTS OF THE TOXICITY EVALUATION OF THE DIALKYL BENZENEDICARBOXYLATE ON THE MAMMALIAN CELL CULTURES AND MICE

R	Phthalates			Isophthalate			Terephthalates		
	Chick Embryo Cells	L-Cells	Mice	Chick Embryo Cells	L-Cells	Mice	Chick Embryo Cells	L-Cells	Mice
CH_3	+	+	+	+	+	+	+	+	+
C_2H_5	+	+	+	+	+	+	+	+	+
n-C_3H_7	+	+	+	+	+	+	+	+	+
iso-C_3H_7	+	+	+	+	+	−	+	+	+
n-C_4H_9	±	+	+	+	+	+	+	+	+
iso-C_4H_9	+	+	±	+	+	+	−	−	±
n-C_5H_{11}	−	±	−	+	+	−	+	+	+
iso-C_5H_{11}	+	+	+	+	+	−	±	+	−
Cyclo-C_5H_9	+	+	±	+	+	+	±	±	±
n-C_6H_{13}	−	−	−	−	−	−	−	−	−
Cyclo-C_6H_{11}	−	−	−	−	−	−	−	−	−
n-C_7H_{15}	−	−	−	−	−	−	+	+	−
Cyclo-C_7H_{13}	+	+	−	+	+	−	−	−	−
n-C_8H_{17}	−	−	−	−	−	−	−	−	−
Cyclo-C_8H_{15}	−	−	...	−	−	...	±	±	...
n-C_9H_{19}	−	−	−	±	+	−	−	−	−
n-$C_{10}H_{21}$	±	+	±	−	−	−	+	+	−
n-$C_{11}H_{23}$	−	−	−	±	±	−	−	−	−
n-$C_{12}H_{25}$	±	+	−	−	−	−	−	−	±

The R_f values for the esters are listed in Table I. A possible occurrence of variation in the position of spots, due to change in the atmosphere of the chamber or the proportion of the solvent, was eliminated by spotting DOP as a reference on the plate along with the esters.

TOXICITY EVALUATION

The toxicity of the esters was evaluated by two methods: (*a*) a cell culture method and (*b*) an animal method. The details of the evaluation of a chemical by cell culture methods have been described previously (5).

The results of both animal and cell culture tests are listed in Table II. It is noteworthy that the results from the two tests coincide within the experimental error. A detailed assessment of this is described further under results and discussion.

EXPERIMENTAL

The infrared spectra were determined with a Perkin-Elmer 337 spectrophotometer. The instrument was calibrated with a polystyrene film and the "normal" slit program was employed throughout the experiments. A KBr disk method was used for solid samples and neat (between NaCl windows) for liquid samples. The recorded wave number values are within ±2 cm.$^{-1}$ of the actual values. The plates for thin-layer chromatography (TLC) were prepared using Brinkmann Silica Gel HF 254 + 366 and were activated at 180° for 30 min. A mixture of hexane–ethyl acetate (90:10) was employed at room temperature as a developing solvent. The solvent front height was maintained at 12 cm. ± 1. Spots were located both by visual examination under a short wave ultraviolet light and by the aid of an indicator, 10% alcoholic solution of phosphomolybdic acid. Elemental analyses[1] were conducted by the Microanalytical Laboratory, Department of Chemistry, University of Texas, Austin, Tex.

General Method of Synthesis of Dialkyl and Dicycloalkyl Benzenedicarboxylate—To 0.2 mole of a dry alcohol was added 0.11 mole of benzene dicarboxylic acid chloride. With the provision for exclusion of water, the mixture was heated under reflux for 4–6 hr. The reaction mixture was examined from time to time by the aid of TLC in order to evaluate the progress of the reaction. Upon completion of the reaction, the content of the flask was poured into a separator containing 100 ml. of diethyl ether. The ether solution was extracted with 20-ml. portions of 1 *N* NaOH solution until the aqueous portion became basic to an indicator paper. The ether layer was washed with water to neutrality, and then was placed in a dry flask containing anhydrous sodium sulfate. After 12 to 24 hr. the ether was filtered and evaporated under dry nitrogen. The residue dialkyl or dicycloalkyl benzenedicarboxylate was obtained in 55 to 75% yield. The purity of the product was verified by TLC (Table I). In a few cases, where the presence of the trace quantity of acid or alcohol was observed, the ester was purified by means of a column packed with a mixture of equal quantities of silica gel and diatomaceous earth,[2] and developed with 200 ml. of *n*-hexane, followed by 300–400 ml. of benzene. The collected fractions, 10–15 ml., were examined by TLC for the presence of the pure ester. In general, the benzene fraction contained the pure ester, which was obtained by the evaporation of benzene *in vacuo*. The main peaks of the infrared spectra of the esters are listed in Tables III, IV, and V.

Toxicity Test—*Mammalian Cell Culture Method*—Monolayers of L-cells and chick embryo cells overlaid with agar were used in this evaluation according to a method reported by Guess *et al.* (5). Several modifications, however, were necessary in

[1] Elemental analysis was done only on those dicycloalkyl benzenedicarboxylates whose structures were not well characterized previously. The results were found to coincide with the calculated values. None was reported in this manuscript.

[2] Marketed as Celite by the Johns-Manville Corp., New York, N. Y.

TABLE III—POSITION OF PEAKS OF THE INFARED SPECTRA OF DIALKYL AND DICYCLOALKYL PHTHALATE[a]

R	νC—H	νC=O	Aromatic Ring Skeletal Vibration	δC—H in Plane	O ‖ νC$_6$H$_5$C—OR Stretching	C—H Out of Plane, Aromatic o-di	Unassigned Peaks
CH_3	3000(w), 2950	1740	1590–1570	1480, 1440	1290–1265(b)	745	1190, 1125(b), 1075, 1045, 960(b), 840(w), 820, 700, 650
C_2H_5	2980	1740	1600, 1570	1450, 1360	1290, 1260(b)	747	1175(w), 1125, 1115–1114(b), 1075, 1045(w), 1015(w), 860(wb), 700
n-C_3H_7	2970, 2880	1740	1600, 1570	1460, 1390, 1350	1290–1265(b)	747	1125, 1075, 1040(w), 965(w), 940, 915(w), 700, 650
iso-C_3H_7	2970, 2910	1725	1590, 1570	1480, 1460–1450(d), 1370, 1350	1290–1275(b)	747	1185, 1145, 1110, 1075, 1040(w), 920, 850(w), 707(w)
n-C_4H_9	2960, 2880	1740	1600, 1580	1470, 1390	1290(b)	748	1135(sh), 1125, 1075, 1040, 945–965(d), 845, 708, 650
iso-C_4H_9	2960, 2880	1740	1600, 1580	1470, 1390, 1370	1290–1260(b)	747	1135(sh), 1120, 1075, 1040, 980, 945(w), 705, 650(w)
n-C_5H_{11}	2955, 2870	1740	1600, 1580	1470, 1385	1290–1260(b)	745	1140–1120(b), 1075, 1040, 965, 875, 705, 650
iso-C_5H_{11}	2955, 2860(w)	1740	1600, 1580	1470, 1390–1370(d)	1285(b)	740	1125(b), 1075, 1040(w), 960–940(d), 700
Cyclo-C_5H_9	2955, 2860	1725	1590, 1570	1480(w), 1440, 1355	1280(b)	740	1170(w), 1140–1125(d), 1070, 1040, 960, 900(w), 850(wb), 708
n-C_6H_{13}	2960–2940(d), 2860	1740	1600, 1585	1480, 1390	1290–1250(b)	745	1140–1120(d), 1075, 1040, 980(w), 910(w), 705, 650
Cyclo-C_6H_{11}	2940, 2860	1725	1600, 1580	1450, 1320(w)	1290–1250(b)	745	1160(w), 1125, 1075, 1040(w), 1015(w), 940(w), 920(w), 920(w), 890(w), 840(w), 708
n-C_7H_{15}	2960–2930(d), 2850	1735	1600, 1580	1470, 1385	1290–1250(b)	745	1140–1120(b), 1075, 1040, 960(wb), 704(w), 650(w)
Cyclo-C_7H_{13}	2930, 2850	1725	1600	1450	1285, 1245	752	1300, 1135–1120(d), 1075, 1035, 970, 880(wb), 840(w), 790, 700, 650, 600, 500
C_8H_{17}	2960–2925(d), 2850	1740	1595, 1580	1485(w), 1460, 1380, 1350	1290–1260(b)	745	1195(w), 1140–1170, 1075, 1040(w), 960(wb), 915(w), 845(w), 700, 650
n-C_8H_{17}	2950–2915(d), 2850	1725	1590, 1570	1460, 1380, 1350	1275(b)	739	1140–1115(d), 1075, 1045, 1035(w), 1000(w), 970(w), 945, 920, 895(w), 875(w), 825(w), 810(w), 795, 700, 650(w), 590(w)
Cyclo-C_8H_{15}	2960(sh), 2920, 2850	1720	1600	1475, 1450	1280, 1245	750	1140–1115(d), 1080, 1035, 1000(w), 970(w), 945, 920, 875(w), 825(w), 810(w), 795(w), 700, 590(w)
C_9H_{19}	2950, 2860	1740	1600, 1580	1470, 1380(w), 1360	1290–1250(b)	742	1200(wb), 1140–1115(b), 1075, 1040(w), 960, 705(w)
$C_{10}H_{21}$	2950(sh), 2915, 2850	1735	1600, 1580	1470, 1375(w)	1290–1270(b)	742	1135(sh), 1125, 1075, 1040(w), 965(wb), 705(w)
$C_{11}H_{23}$	2950(sh), 2915, 2850	1735	1600, 1580	1465, 1375(w)	1290–1270(b)	742	1140–1120(b), 1075, 1040(w), 960(wb), 705(w)
$C_{12}H_{25}$	2950(sh), 2920, 2850	1740	1600, 1580	1470, 1380(w)	1290–1270(b)	742(w)	1135(sh), 1120, 1075, 1040(w), 950(wb)

[a] Key: w, weak; d, doublet; b, broad; sh, shoulder; wb, weak broad. Solvent: the spectra of all the esters were determined neat except R = CH_3 and cyclo C_8.

the method to make it suitable for the samples tested in this investigation. If the esters were crystalline, approximately 20 mg. of each was placed directly on the agar overlay. For liquid esters, 35 mg. of each was applied directly to a paper disk, 12.7 mm. diameter, which had previously been placed on the agar overlay. The culture plates were then incubated for a period of 24 hr., at which time the cells adjacent to the test samples were observed for possible toxicity. If the compound affected the cells by creation of zone of dead cells (inhibition zone) it was assumed to be toxic [designated by a positive (+) sign]. If the cells remained intact, the compound was assumed to be nontoxic [negative (−) sign]. In those cases where the results appeared in doubt, pending further tests, the results were identified with a positive–negative sign (±). These results are shown in Table II. Each of the compounds were tested a minimum of 3 times.

Intraperitoneal Mice Method—Each of the compounds was administered i.p. in groups of 3 mice at a concentration level of 5 mmoles/Kg. in either cottonseed oil or in castor oil, depending upon the solubility of the specific compound. The animals were kept under observation for a period of 4 days or until death. In general, the esters exerted a depressant effect in mice and induced observable ptosis. Gross toxicity results based upon death are included in

TABLE IV—THE POSITION OF PEAKS OF THE INFRARED SPECTRA OF DIALKYL AND DICYCLOALKYL ISOPHTHALATES[a]

R	νC—H	νC=O	Aromatic Ring Skeletal Vibration	δC—H in Plane	$\nu C_6H_5C(=O)—OR$ Stretching	δC—H out of Plane Aromatic *m*-di	Unassigned Peaks
CH_3	3120(d), 2950	1730	1600, 1580	1480, 1460, 1445–1430(d)	1290, 1250(b)	825(w), 808(w), 725	1315, 1190, 1130, 1095, 1075, 1000(w), 980, 955(d), 930, 925, 865, 670, 655
C_2H_5	2980, 2900(w)	1735	1605(w)	1475, 1450, 1390(w), 1370	1285(w), 1240(b)	825(w), 735(d)	1310, 1170, 1135, 1100, 1080, 1025, 930(d,b), 865(w), 658(w)
n-C_3H_9	2970, 2880	1735	1605(w)	1460, 1440, 1390, 1350(w)	1240	735(d)	1305, 1165, 1135, 1100, 1080, 985(w), 960(b)
iso-C_3H_7	2985, 2940(w)	1735	1610(w)	1475, 1440, 1380–1360(d)	1285(w), 1245(b)	830, 730(d)	1305, 1180, 1165(w), 1145, 1110, 1075, 940, 915(w), 890(w), 650
n-C_4H_9	2960, 2940(w), 2870	1730	1600(w)	1460, 1430(w), 1380	1245(b)	730(d)	1300, 1185(w), 1165(w), 1135, 1095, 1080, 970–950(d,b)
iso-C_4H_9	2960, 2870(w)	1730	1600(w)	1460, 1430(w), 1390–1370(d)	1240(b)	730(d)	1300(d), 1170, 1130, 1092, 1075, 990, 940(w), 820(w)
n-C_5H_{11}	2950, 2920, 2850	1725	1600(w)	1465, 1435, 1380	1235	730(d)	1300, 1165(w), 1135, 1095, 1075, 975
iso-C_5H_{11}	2960, 2870	1730	1605(w)	1465, 1435(w), 1385–1370(d)	1235(b)	830(w), 730(d)	1300, 1170, 1135, 1098, 1080, 975(b)
Cyclo-C_5H_9	2960, 2870	1730	1605(w)	1440, 1360, 1325	1240	835(w,b), 1730(d)	1300, 1190(w), 1165, 1135, 1098, 1075, 1030(w), 960, 690
n-C_6H_{13}	2960, 2935, 2865	1740	1605(w)	1470, 1435(w), 1390	1240	830(w), 735(d)	1300, 1165(w), 1140, 1100, 1080, 900
Cyclo-C_6H_{11}	2940, 2860	1725	1605(w)	1450	1240	830(w), 730	1300, 1160(w), 1140, 1120, 1098, 1080, 1040, 1015, 960(w), 890(w)
n-C_7H_{15}	2960–2930(d), 2850	1725	1605(w)	1465, 1435(w), 1380	1235(b)	830(w), 735	1300, 1165(w), 1135, 1098, 1080
Cyclo-C_7H_{13}	2940, 2860	1725	1605(w)	1460	1280(w), 1240	820(w), 730	1300, 1160(w), 1135, 1095, 1075, 1015(w), 1000(w), 975, 885(w)
n-C_8H_{17}	2960(sh), 2935, 2860	1735	1605(w)	1470, 1440(w), 1380(w)	1235(b)	730	1305, 1165(w), 1135, 1095, 1075, 960(w,b)
Cyclo-C_8H_{15}	2960(sh), 2920, 2840(w)	1720	1600(w)	1475, 1450, 1375(w)	1275, 1240	750	1140, 1120, 1080, 1035, 1000(w), 970(w), 945, 920, 875(w), 700
n-C_9H_{19}	2960(sh), 2940, 2860	1740	1605(w)	1470, 1440(w), 1380(w)	1235	730	1305, 1135, 1098, 1075, 970(w,b)
n-$C_{10}H_{21}$	2950(sh), 2925, 2850	1740	1605(w)	1470, 1380(w)	1235	730	1305, 1130, 1095, 1075, 970(w,b)
n-$C_{11}H_{23}$	2940(sh), 2915, 2840	1735	1605(w)	1465, 1385(w)	1240	730	1305, 1160(w), 1135, 1095, 1075, 970(w,b)
n-$C_{12}H_{25}$	2950(sh), 2920, 2850	1735	1605(w)	1465, 1385(w)	1235	730	1305, 1160(w), 1130, 1095, 1075

[a] Key: same as in Table III.

Table II. A negative sign (−) indicates no deaths, a positive sign (+) 2 or 3 deaths, and a positive-negative sign (±) only one death.

DISCUSSION

Infrared—The major peaks of the I.R. spectra of dialkyl and dicycloalkyl benzenedicarboxylates, together with the spectra of phthalic acid, phthalic anhydride, and isophthalic and terephthalic acid, are listed in Tables III–VI. The peaks for stretching frequencies of C=O and of C—H for acids and their appropriate esters occur in different regions of the infrared spectrum. The presence of phthalic acid, a hydrolysis product of a dialkyl phthalate, can be verified by the presence of C=O band at 1700 cm.$^{-1}$ next to a conjugated ester C=O band at 1740 cm.$^{-1}$. This distinction, however, cannot be made if the quantity of acid to ester is less than 5%—only the peak for ester C=O will be observed. The presence of phthalic anhydride can be identified in a like manner by the presence of C=O bands at 1760 and 1855 cm.$^{-1}$. The bands in the fingerprint region, which are characteristic of a given compound, are further means of identifying an unknown molecule, providing the spectrum of the authentic sample is available. The latter statement, however, is not quite applicable in those cases where the compound ought to be differentiated from its closely related homologs.

The esters of the three isomeric benzenedicarboxylic acids can be differentiated (*a*) on the basis of peak positions for carbonyl frequency, 1710–1745 cm.$^{-1}$; (*b*) aromatic ring skeletal vibration, 1500–1605 cm.$^{-1}$; (*c*) in-plane C—H bending, 1350–1490 cm.$^{-1}$; (*d*) $C_6H_5—\overset{O}{\overset{\|}{C}}—OR$ stretching vibration, 1230–1290 cm.$^{-1}$; and (*e*) out-of-plane aromatic C—H bending vibration, 720–840 cm.$^{-1}$. In general, the stretching frequency for C=O of

TABLE V—THE POSITION OF PEAKS OF THE INFRARED SPECTRA OF DIALKYL AND DICYCLOALKYL TEREPHTHALATE[a]

R	νC—H	νC=O	Aromatic Ring Skeletal Vibration	δC—H in Plane	O ‖ νC$_6$H$_5$C—OR Stretching	δC—H out of Plane Aromatic *p*-di	Unassigned Peaks
CH_3	3000(w), 2950(w)	1725, 1700(sh)	1575, 1505	1440, 1410, 1380(w)	1280	815, 790, 735(w)	1192, 1130, 1110, 1020, 955, 880, 800(w), 695(w), 550(w), 500(w)
C_2H_5	2990, 2940, 2905(w)	1730	1570(w), 1500	1480, 1450, 1410, 1370	1275	840, 810(w), 725	1130, 1100, 1020, 870, 500, 450
n-C_3H_7	2970	1725	1570(w), 1500(w)	1475, 1410, 1390(w), 1350(w)	1270, 1250(sh)	732	1195, 1120–1100(d), 1020, 940, 875, 700(w)
iso-C_3H_7	2980, 2920(w)	1720	1500(w)	1470, 1410, 1380(d), 1350(d)	1275	845, 740	1185, 1130, 1100, 1015, 920(w), 880(w)
n-C_4H_9	2960, 2930, 2875	1735	1575(w), 1500(w)	1470, 1410, 1380	1280, 1270(b)	835(w), 790(w), 725	1115–1100(d), 1018, 960–940(d), 870
iso-C_4H_9	2960, 2860	1720	1500	1475, 1400, 1375	1270, 1250	800, 730	1135, 1120, 1105, 1015, 980, 948, 900, 870, 500, 430
n-C_5H_{11}	2960, 2935, 2865	1735	1580, 1500	1460, 1410, 1380	1270, 1250(sh)	735	1120–1105(d), 1020, 970(wb), 875(w)
iso-C_5H_{11}	2950, 2870	1725	1580(w), 1500	1470, 1410, 1390, 1370(d)	1270, 1250	820(w), 730	1120–1105(d), 1020, 965(b), 875
Cyclo-C_5H_9	2960, 2870(w)	1715	1505	1440, 1410, 1365, 1330(w)	1265, 1250(sh)	735	1330(w), 1165, 1120, 1105, 1015, 960(w), 880(w)
n-C_6H_{13}	2935(d), 2850(d)	1715	1500(w)	1460, 1400, 1370(w)	1270	840, 730	1135(w), 1120, 1105, 1020, 955(b), 875(w)
Cyclo-C_6H_{11}	2930, 2850	1730, 1710	1500	1450, 1400, 1370	1270, 1250	835, 735	1155(w), 1120, 1105, 1040, 1015, 940, 910(w), 895, 880, 510, 450
C_7H_{15}	2945(sh), 2915, 2845	1730	1500	1470, 1410, 1390	1270, 1245(sh)	839, 725	1195(w), 1120, 1105, 1040(w), 1018, 985(w), 920(w), 870, 500
Cyclo-C_7H_{13}	2945(sh), 2920, 2855	1715	1500	1470, 1450, 1410, 1375(w)	1270	839, 820(w), 760(w), 730	1325, 1125, 1100, 1015, 975, 905(w), 872, 500
C_8H_{17}	2960, 2920, 2850	1720	1675, 1505	1480, 1470, 1410, 1380, 1350(w)	1280	840, 730	1320, 1135, 1125, 1105, 1040(w), 1020, 875, 500(w), 470(w), 435(w)
Cyclo-C_8H_{15}	2925, 2850	1710	1500(w)	1470, 1450, 1410, 1375(w)	1270, 1255, 1220	845, 820, 810, 740(d)	1330, 1140, 1125, 1105, 1035, 1015, 945(w), 920(w), 880(w), 870(w)
n-C_9H_{19}	2950(w), 2910, 2850	1725	1500	1470, 1400, 1380	1275	830(w), 728	1125(d), 1100, 1020, 950(w), 900(w), 870(w), 500(w)
$C_{10}H_{21}$	2900, 2840	1710	1500(w)	1475–1460(d), 1400, 1375(w)	1285, 1270	840, 740	1135, 1125, 1110, 1020(w), 1000(w), 960(w), 875
$C_{11}H_{23}$	2915, 2850	1730	1500(w)	1475, 1450(w), 1405, 1390	1275, 1250	825(w), 730	1130, 1105(w), 1070(w), 1020, 975(w), 930(w), 875, 500(w)
$C_{12}H_{25}$	2950, 2915, 2850	1720	1500(w)	1475(d), 1400(w)	1290, 1280, 1255	845(w), 730	1320(w), 1140, 1130, 1110, 1025, 960(w), 875(w), 500(w)

[a] Key: same as in Table III.

TABLE VI—THE POSITION OF PEAKS OF THE INFARED SPECTRA OF BENZENEDICARBOXYLIC ACIDS AND PHTHALIC ANHYDRIDE[a]

	νCH	νC=O	Aromatic Ring Skeletal Vibration	δCH in Plane	O ‖ νC$_6$H$_5$C—OH	δC—H out of Plane, Aromatic	Unassigned Peaks
Phthalic acid	3100–2850(b)	1695	1590	1490(w), 1410	1280	740	1150(w), 1075, 1005(w), 975(w), 910(wb), 830(w), 800, 675, 555
Isophthalic acid	3070–3000(b)	1695	1610–1585	1430	1290–1280(b)	730	1160(w), 1095(w), 1075(w), 930–920(b), 830(w), 690
Terephthalic acid	3100–2900(b)	1690	1570(w), 1500	1420	1275	730	1200, 1130, 1110, 1070, 930(b), 880, 780, 695
Phthalic anhydride	3075–3050	1850, 1765	1600, 1515(w)	1475, 1390, 1360	1255	715	1165, 1105, 1070(w), 1005, 905, 840, 800, 675, 640, 535

[a] Key: same as in Table III.

phthalates are slightly higher than isophthalates and terephthalates. The field effect of the proximately located C=O group may be a reason for observing a higher C=O stretching frequency for phthalates.

The doublet for aromatic ring skeletal vibration at 1580–1600 cm.$^{-1}$ observed in the spectra of a phthalate is absent in the spectra of iso- and terephthalates. Instead there is a single band at 1605 cm.$^{-1}$ for iso- and two bands at 1570 (weak) and 1500 cm.$^{-1}$ for terephthalates.

The bands due to the in-plane bending vibration, 1350–1490 cm.$^{-1}$, quite often vary somewhat among the esters of the three benzenedicarboxylic acids. The variation in this region, however, is not sufficient to lead one to an unequivocal identification of an unknown ester; a reference to the spectrum of an authentic sample is necessary.

Out-of-plane bending bands, 700–900 cm.$^{-1}$, are characteristic of the esters of each of the three isomeric acids. Positional isomers of disubstituted benzene usually contain I.R. bands which are at 690–840 cm.$^{-1}$.

$$C_6H_5—\overset{\overset{O}{\|}}{C}—OR$$

The stretching band for C_6H_5—COR for phthalates is quite close to terephthalates at 1275–1280 cm.$^{-1}$. This band for isophthalate comes at 1235–1240 cm.$^{-1}$ and can serve for identification purposes.

Finally, the position of the unassigned peaks in the tables could function as fingerprints for detection and differentiation of the esters of the three isomeric acids. Various esters of a given acid, such as phthalic acid, can also be discerned if the molecular weight of the homologs are not too close.

Thin-Layer Chromatography—TLC proved to be an excellent and rapid method for the detection of the product of hydrolysis of an ester or the presence of other esters and the parent acid and alcohol mixed with a given ester. The R_f values for the esters of the three isomeric benzenedicarboxylic acids are listed in Table I. In general, the R_f value in this series increases from *ortho* to *meta* to *para* isomer. Apparently, the R_f value in the hexane–ethyl acetate developer for a given molecular weight of these esters is inversely proportional to their dipole moment value. Cyclic compounds show lower R_f values as compared to their straight chain counterpart which, in a sense, indicates the influence of polarity of a compound on its R_f value. Since only a fraction of a mg. is necessary to identify a compound by TLC, this technique in conjunction with I.R. manifests a great potential in the detection and identification of plasticizers leached out of a plastic. Such identification, however, may become laborious if some esters unrelated to benzenedicarboxylate are in the mixture.

Toxicity—As is depicted in Table II, toxicity results obtained by the cell culture method quite frequently coincide with the toxicity evaluation conducted on mice.

In general, low molecular weight dialkyl benzenedicarboxylates (alkyl group C1 to C5) manifest toxic effect. This toxic effect could be ascribed to the higher degree of solubility of the low molecular weight esters, as compared with their higher homologs. However, since both di-*n*-decyl phthalate and di-*n*-decyl terephthalate show toxicity to cell cultures, the conclusion based merely on relating the toxicity of a compound in the series to its solubility or molecular weight becomes subject to some doubts. It is noteworthy that, unlike a freshly synthesized pure di-*n*-decyl phthalate, the commercial sample of this compound was found to be extremely toxic to mice. Although determining the toxicity of a commercial plasticizer is beyond the scope of this investigation, the importance of purity and stability of a compound should not be ignored. Apparently identical plasticizers obtained from various sources or subjected to various conditions during the process of incorporation into a polymer may manifest quite different toxic effects.

The question of whether toxicity effects of the esters are due only to their degradation products rather than to the intact ester molecule has been examined in the past with citrate plasticizers (6), where toxicity proved to be due to the ester, despite the fact that citric acid *per se* was also toxic. In the dialkyl benzenedicarboxylates, although their products of hydrolysis were found to be toxic, evaluation of toxicity data suggested that these esters, as citrates, exert their toxic effects as the intact ester molecule. The molecules, di-*n*-propyl, di-iso-propyl, di-*n*-butyl, di-iso-butyl, di-*n*-pentyl, and di-iso-pentyl, all show similar ratings in the toxicity evaluation, whereas the rate of hydrolysis of the iso derivatives would be expected to be lower than the *n* derivatives. Likewise, no great difference, if any, would be expected in the rate of hydrolysis of di-*n*-pentyl and its homolog di-*n*-hexyl phthalates, but biologically these two behave differently. As indicated above, phthalates with alkyl groups C_6 to C_9 do not show a toxic effect on the cell.

The reason for the toxic manifestation of di-*n*-decyl phthalate and terephthalate is not yet clear. Such behavior, however, could be postulated to be due to the inhibition of the function of certain enzyme systems, whose metabolites possess a structure analogous to that of the ester.

The mice tests were included only as another screening method to observe if the results noted in the cell tests could be manifested in animals. Keeping in mind the limitation in the mouse test, it is still interesting to note that a good correlation exists between the results shown for the mammalian cell method and for the i.p. mouse test. Further animal studies would, of course, be needed to establish true toxicity patterns based upon definite dose levels of the compounds. It is, however, important to state that the close correlation between the animal test and cell test result renders the latter an appealing method due to its inherent convenience, rapidity, and economy.

In conclusion, perhaps the important point to be gained from this study is that greater attention should be paid to the actual composition of the plasticizer which is to be used for a medical application. A pure plasticizer may have no demonstrable toxic response, but the presence of related homologs and isomers could add a toxic potential to the plasticizer. To this possibility one must also include the potential toxic effects from degradation products present or forming in the plasticizer during manufacture of a plastic device, or occurring after the device is stored under various conditions for undetermined periods of time. Good quality control procedures in the manufacture of a medical device should prevent the entrance of potentially toxic

plasticizers to the medical and para-medical professions.

REFERENCES

(1) Lawrence, W. H., Mitchell, J. L., Guess, W. L., and Autian, J., *J. Pharm. Sci.*, **52**, 958(1963).
(2) Rosenbluth, S. A., Weddington, G. R., Guess, W. L., and Autian, J., *ibid.*, **54**, 156(1965).
(3) Meyers, D. B., Autian, J., and Guess, W. L., *ibid.* **53**, 774(1964).
(4) Drug-Plastic Research and Toxicology Laboratories, College of Pharmacy, University of Texas, Austin, Tex., unpublished data.
(5) Guess, W. L., Rosenbluth, S. A., Schmidt, B. C., and Autian, J., *J. Pharm. Sci.*, **54**, 1545(1965).
(6) Rosenbluth, S. A., personal communication, 1966.

Synthesis of Tropine-Labeled Atropine II

Prototype Synthesis for the Preparation of Tropine-^{14}C and Atropine-^{14}C from Arabinose-^{14}C

By GILBERT C. SCHMIDT*, THOMAS E. ELING, JOHN M. McOWEN, and JOHN C. DRACH†

Prototype methods for the synthesis of tropines-^{14}C and tropine-labeled atropines are described. These compounds and the requisite labeled intermediates may be synthesized from arabinose-5-^{14}C or arabinose-UL-^{14}C. Yields for each step in the conversion of arabinose to succindialdehyde *via* 2,5-diethoxytetrahydrofuran have been determined. Predicted yields of 27 per cent atropine, based on starting pentose, were confirmed by synthesis of the alkaloid from 2 mmole quantities of arabinose. This procedure is the first published method for labeling atropine in the carbon skeleton of the tropine moiety.

FEW METHODS for selectively labeling atropine have been published. Labeled tropic acid has been synthesized by adaptations of Blicke's procedure (1), then converted to correspondingly labeled atropine by modifications of the Wolffenstein esterification (2). Using these methods, atropine has been labled with ^{14}C in the carboxyl position (3) and in the α-position (4) of the tropic acid moiety. Subsequent studies with the labeled alkaloids (5–9) have contributed much to an understanding of atropine metabolism, but the metabolic fate of the tropine moiety is not known.

The virtual absence of studies concerned with the metabolic fate of tropine indicates that suitably labeled compounds are not readily available for study. Randomly ^{14}C-labeled atropine has been biosynthesized (10) and atropine has been randomly labeled with tritium (11), but these compounds lack the desired specificity of labeling. Selective tritiation of tropine has been accomplished recently (12), but the heterocycle was converted to deptropine rather than atropine (13). *Datura metel* is known to incorporate 80–85% of the radioactivity from acetate-2-^{14}C into positions 2,3,4 of tropine (14, 15), but the exact position or positions of labeling are unknown. When *D. metel* is grown on acetate-1-^{14}C (14, 15) or *Datura stramonium* is grown on ornithine-2-^{14}C (16, 17), radioactivity is incorporated with stereochemical specificity into position 1 or position 5 of the tropine moiety. These biosynthetic compounds have not been available for metabolic studies and problems inherent in biosynthetic methods limit feasibility of this approach to tropine labeling. Standard synthetic methods for labeling the tropine moiety of atropine are needed, but few have been reported. Werner *et al.* (3), Fodor *et al.* (18), and Eling *et al.* (19) have used classical organic syntheses to obtain *N*-methyl-^{14}C-tropine and *N*-methyl-^{14}C-atropine, but these compounds are of limited value because the methyl carbon attached to the endo nitrogen bridge, rather than the carbon skeleton of tropine, is labeled. The need of practical methods for labeling the carbon skeleton of tropine is acute and the problem remains unsolved.

Three fundamental problems are encountered in the synthesis of tropine-labeled atropine: reproducibility of the esterification, feasibility of the Robinson condensation, and availability of labeled intermediates suitable for synthesis of succindialdehyde and acetone dicarboxylic acid, the

Received May 15, 1967, from the * Children's Hospital, and the Departments of Biological Chemistry and Pediatrics, Division of Pharmacology, University of Alabama Medical Center, Birmingham, AL 35233, and the College of Pharmacy, University of Cincinnati, Cincinnati, OH 45221
Accepted for publication July 5, 1967.
Presented in part to the Pharmacology and Biochemistry Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.
This investigation was supported by grant GM-13844 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md., and grant AFOSR 87-65,66 from the U. S. Department of the Air Force, Washington, D. C.
Abstracted in part from a thesis submitted by Thomas E. Eling to the Graduate School, University of Alabama, Birmingham, in partial fulfillment of Doctor of Philosophy degree requirements.
Previous paper: Schmidt, G. C., Eling, T. E., and Drach, J. C., *J. Pharm. Sci.*, **56**, 215(1967).
† Present address: Research Division, Parke-Davis and Co., Detroit, MI 48232