Potential Antifertility Agents. 6. Synthesis and Biological Activities of Optical Isomers of 4β-(p-Methoxyphenyl)-2β-methylcyclohexane-α-carboxylic Acid and Related Compounds[1]

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Syntheses and biological activities are reported for (+) and (−) isomers of 4β-(p-methoxyphenyl)-2β-methylcyclohexane-α-carboxylic acid and related cyclohexene acids. The approximately equal uterotropic activity seen with the optical isomers of the title compound is interpreted in terms of an estrogenic receptor model in which there is proposed a new spatial area which may be involved in the elicitation of a uterotropic response.

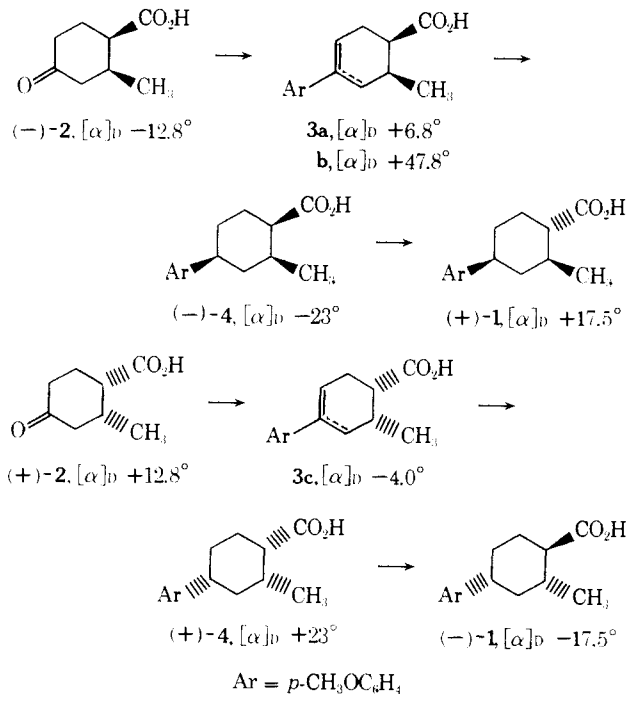
Recently, we reported a series of compounds which had a large separation between uterotropic and hypocholesterolemic activities.[2] The compound chosen for more extensive studies was racemic 4β-(p-methoxyphenyl)-2β-methylcyclohexane-α-carboxylic acid [(±)-1] for which evidence was presented that the biological activity seen with the compound was resultant from its stereochemical relationship with steroidal estrogens. It has been reported that the unnatural (−) enantiomer of estradiol retains 90% of the hypocholesterelemic activity of (+)-estrone having the natural configuration, yet possesses only 0.06% of the uterotropic activity of (+)-estrone.[3] We therefore predicted that an analogous relationship should exist between the 1α,2β,4β and 1β,2α,4α optical isomers of 1 which correspond in configuration to (+)- and (−)-estradiol, respectively. Thus, we expected the 1β,2α,4α isomer to show an even greater separation between uterotropic and hypocholesterolemic activities than seen with (±)-1. We now report the preparation and biological activities of the optical antipodes (+)- and (−)-1 and discuss implications of their biological activity in terms of structural requirements at the receptor level. It should be noted that use of the α and β designations herein refers to depiction of the structures as shown in Scheme I so that carbons 1, 2, and 4 of the cyclohexane ring correspond to carbons 13, 14, and 9, respectively, of the steroidal skeleton.

Chemistry. A number of attempts to resolve (±)-1 *via* fractionation of asymmetric amine salts were unsuccessful. Although several asymmetric amines gave salts with (±)-1 which were recrystallized to constant melting point and rotation, the free acids obtained after liberation from salts were not optically pure. As an alternative approach to resolution, we investigated various esters of (±)-1 with asymmetric alcohols such as (−)-menthol, methyl (−)-mandelate, (−)-2-octanol, testosterone, and methyl (−)-α-hydroxy-α-trifluoromethylphenylacetate. This approach provided not only a potential method of resolution but also a quantitative method of assay for optical purity of a sample thought to have been resolved by another method if the two diastereoisomeric esters formed could be distinguished by glpc or nmr. The pure (−)-1 isomer was obtained from this approach by fractionation of the esters formed from (±)-1 and testosterone. Although successful, this method was impractical for providing quantities of antipodes of 1 desired for biological evaluation. A very feasible approach appeared to be synthesis of the enantiomers of 1 starting with the optical antipodes of the keto acid 2 and elaboration *via* the stereospecific synthesis[2] abbreviated in Scheme I. This approach would not only provide enantiomeric forms of 1 but also chiral forms of 3, the racemate of which has biological activity similar to that of (±)-1.[2]

Attempts to form crystalline salts between (±)-2 and dehydroabietylamine or (−)-α-(1-naphthyl)ethylamine failed. Surprisingly, diastereoisomeric esters from (±)-2 and the optically active alcohols mentioned above were indistinguishable in several glpc and tlc systems tried. Additionally, ester formation and/or attempted fractionation in some instances led to epimerizition to the trans isomer of 2. Because of these difficulties, use was made of commercial facilities† for resolution of 2. Samples of (+)- and (−)-2 provided to us were shown to be optically pure by nmr analysis of diastereoisomeric esters formed with methyl (−)-mandelate (*cf.* Experimental Section).

The two optical antipodes of 2 were elaborated as indicated in Scheme I to optically active forms of 3, 4, and 1. Two forms of 3 (**3a** and **3b**) from (−)-2 were isolated. The only chemical difference between 3a and **3b** is the ratio of Δ^4:Δ^3 isomers (approximately 2.5 and 1.4 for 3a and **3b**, respectively). Samples of 3a and **3b** each were hydrogenated separately to give samples of (−)-4 of essentially the same specific rotation. Therefore, the pure Δ^4 and Δ^3 isomers of 3 must differ greatly in their rotations at the D wavelength. Specific rotations of (−)-1 obtained by synthesis according to Scheme I agreed with those obtained from resolution of (±)-1 *via* the testosteronyl ester. Optical purity of 1 was confirmed by nmr studies employing methyl (−)-mandelate (*cf.* Experimental Section).

Scheme I



†Norse Laboratories, Santa Barbara, Calif. 93103. From 260 g of (±)-2, 10 g of (+)-2 and 15.6 g of (−)-2 were provided to us. It was stated by Norse Laboratories that the resolution was tedious and resulted in low yields.

Table I. Biological Properties of Enantiomeric Forms of Acids 1 and 3

A. Uterotropic Activities (Rats)[a]

Compd	Total dose, μg	Uterine wt increment over control, mg[b]
3a	250	+18.0
	2500	+32.9
3b	250	+15.3
	2500	+28.3
3c	250	+43.1
	2500	+59.8
(±)-**3**	250	+21.9
	2500	+39.7
(+)-**1**	250	+28.2
	2500	+57.2
(−)-**1**	250	+29.3
	2500	+44.5
(±)-**1**	250	+29.4
	2500	+53.9
EE[c]	0.2	+17.0
	2.0	+73.2

B. Hypocholesterolemic Assays[a,d]

Compd	Dose, mg/kg/day	Serum cholesterol, % change
3a	5	−32
3b	10	−46
3c	5	−65
(±)-**3**	5	−64
(+)-**1**	10	−60
(−)-**1**	10	−66
(±)-**1**	10	−65

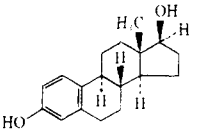
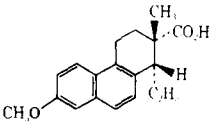
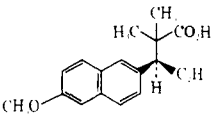
[a] *Cf.* ref 2 for description of assays. [b] Vehicle control uteri typically weighed 20–25 mg. [c] Ethinylestradiol. [d] Compounds assayed at twice the minimal effective hypocholesterolemic doses of the corresponding racemates (*cf.* ref 2) with the exception of 3b which showed insignificant lowering at 5 mg/kg. All values shown were statistically significant at 95–99% confidence levels.

Assignments of absolute configuration depicted in Scheme I must be considered provisional. Assignments were made by inferring that **3c**, which showed greater estrogenicity than **3a** or **3b**, has absolute configuration corresponding to that of the biologically active *l-cis*-bisdehydrodoisynolic acid.[4]

Biology. Methodology for the biological assays has been described previously.[2] All data are from oral dosing of aqueous solutions of sodium salts of the acids. Results are summarized in Table I. It will be noted that the levorotatory form **3c** has significantly greater uterotropic activity than either dextrorotatory mixture **3a** or **3b**, with the racemic form (±)-**3** showing intermediate potency. The hypocholesterolemic activities of the optical isomers of **3** generally paralleled their estrogenic potencies.

The optical antipodes, (+)- and (−)-**1**, showed essentially no difference in hypocholesterolemic activities as had been predicted. On the other hand, contrary to our predictions, little or no differences were seen between uterotropic potencies of (+)-**1** and (−)-**1**. The dextrorotatory isomer (+)-**1** showed slightly greater uterotropic activity at the 2500-μg dose than its enantiomer, but certainly the vast differences which had been predicted were not seen.

Table II. Relative Uterotropic Potencies between Enantiomeric Forms of Various Estrogens and of 4β-(*p*-Methoxyphenyl)-2β-methylcyclohexane-α-carboxylic Acid (**1**)

Compd	Absolute confign of more active enantiomer	Ratio of uterotropic activities between enantiomers[a]
Estradiol		10,000:1
Fenocyclin		200:1
Methallenestril		5:1
1		1:1

[a] Data for the first three compounds are from ref 5.

Discussion

It is interesting to compare differences in biological activity between pairs of optical isomers of known potent estrogens and of **1** (Table II). Looking at the first three compounds in Table II, all of which are potent estrogens, one sees a trend that as structures become less complex, the differences between biological activities of their optical antipodes decrease. Thus, natural (+)-estradiol with five asymmetric carbons is 10,000 times as uterotropic as its enantiomer; the (−) isomer of fenocyclin, having only two asymmetric carbons, is 200 times as uterotropic as its (+) enantiomer; the (−) enantiomer of methallenestril, having only one asymmetric carbon, is but 4–5 times as uterotropic as its (+) antipode.[5] With **1**, which has three asymmetric carbons, we had expected a considerable difference between activities of its enantiomers analogous to that seen with fenocyclin or estradiol. This was particularly true since in earlier work we had hypothesized that the activity seen with (±)-**1** was resultant from its interaction, albeit weak, with estrogenic receptors. The approximately equal potencies seen with (+)- and (−)-**1** at first glance appeared to refute this hypothesis.

Since (±)-**1** is very weak in estrogenic potency, *viz.* up to 2500 times less active than steroidal estrogens,[2] one could view the equal activities seen with its enantiomers as being consistent with Pfeiffer's hypothesis which states that the activity difference between antipodes decreases with decreasing potencies of the drugs.[6] The Pfeiffer relationship appears to be invalid, however, for methallenestril, as pointed out by Terenius,[5] since the uterotropic activity of methallenestril is of the same order of magnitude as fenocyclin; yet the antipodes of methallenestril show only a four- to fivefold difference in activity. On the other hand, it has been questioned whether the relatively high uterotropic activity seen with the (+) antipode of methallenestril may be due to a partial racemization in the biological organism of the (−) antipode, a process which according to Gay and Horeau might be more easily effected than a racemization of (−)-fenocyclin which possesses two asymmetric carbons.[7]

We feel that a better explanation to account for the ac-

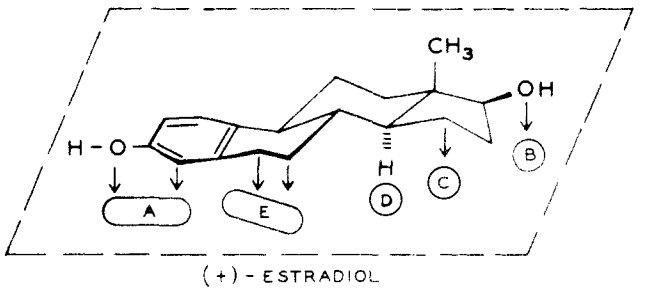


Figure 1. Hypothetical model of estrogenic receptor site depicting "fit" of (+)-estradiol with proposed areas A-E involved in elicitation of biological response.

tivity differences between (+)- and (−)-methallenestril, as well as activities seen with other compounds including (+)- and (−)-1, is provided by a working model of an estrogenic receptor which we have adopted (Figure 1). For a potent estrogenic response (*cf.* estradiol model), a number of crucial sites must be activated, among them a site A for a hydroxylated aromatic moiety, a site B for an oxygenated function, and sites C and E for alkyl groups, as well as sites that accommodate the steroidal ring C or a portion thereof. Seco steroids such as various *trans*-doisynolic acids[4,8] and 4-(*p*-methoxyphenyl)-3-ethyl-2-methyl-Δ^3-cyclohexenecarboxylic acid,[9] adequately cover these sites and indeed are potent estrogens. An additional site D must be present in which axial methyl or ethyl groups can be accommodated. A site D is involved, in lieu of C, in various biologically active doisynolic acids of the cis configuration[4] (*e.g.*, fenocyclin) and in the 1α,2α,4β isomer corresponding to (+)-1.[2] In this model, the ethyl group in (−)-methallenestril would fit the site C, while in the (+) form it complexes with site D (*cf.* Figure 2); hence, both enantiomers elicit a potent response. The enantiomers of the more rigid structures (+)-estradiol and (−)-fenocyclin can no longer fill the crucial sites A-E and, hence, are vastly less potent than the antipodes of the natural configuration. A compound such as (+)-1 can activate A, B, and C but lacks an ethyl moiety at position 3 required for potent activity. The compound is thus only weakly estrogenic, but the response is nevertheless measurable, and indeed the corresponding (±)-1β,2β,4β and (±)-1α,2β,4α isomers which cannot complex A, B, and C inclusive are devoid of activity.[2] We thus analogously expected that (−)-1 would be inactive in the uterotropic response relative to (+)-1. The fact that (+)- and (−)-1 are of approximate equal uterotropic potency suggests to us that *there exists on the estrogenic receptor a complimentary site C′ that can accommodate an alkyl group for elicitation of a response.* This is illustrated in Figure 2 in which (−)-1 is depicted in a manner in which it complexes with sites A, B, and C′. That is, carbons 1, 2, and 4 of the cyclohexane moiety in (−)-1 may be viewed as corresponding *not* to carbons 13, 14, and 9, respectively, of the steroidal skeleton [as (−)-1 is drawn in Scheme I], but rather to steroidal carbons 13, 12, and 9. Thus, the fact that equal activities are seen with (+)- and (−)-1 does not disprove our original hypothesis that (±)-1 derives its activity from complexation with estrogenic receptors, but rather broadens our view of stereochemical requirements at the receptor level. It is of interest to compare our proposed receptor model with that recently proposed by Weber and Galantay for steroidal estrogens.[10] They suggest that for estrogenic activity, an area above rings A, B, and part of C of the steroidal skeleton be free of projecting alkyl groups.

As an incidental point it should be mentioned that RO in the figures shown most likely represents a phenolic function since studies have suggested that *in vivo* demethylation of fenocyclin, methallenestril, and 3-*O*-methyl ethers of steroidal estrogens is necessary for biological response.[11] We point out also that although the figures imply that areas such as C′ and D are in the same single receptor site (one of many on a gross protein surface), this may be an oversimplification. For example, (+)-methallenestril and (−)-1 may be binding to the same gross receptor protein as their "natural" configuration antipodes but to different areas along its surface. Nevertheless, the proposed model does provide a useful framework for further experiments. We thus have adopted as a working hypothesis that the uterine estrogenic receptor can accom-

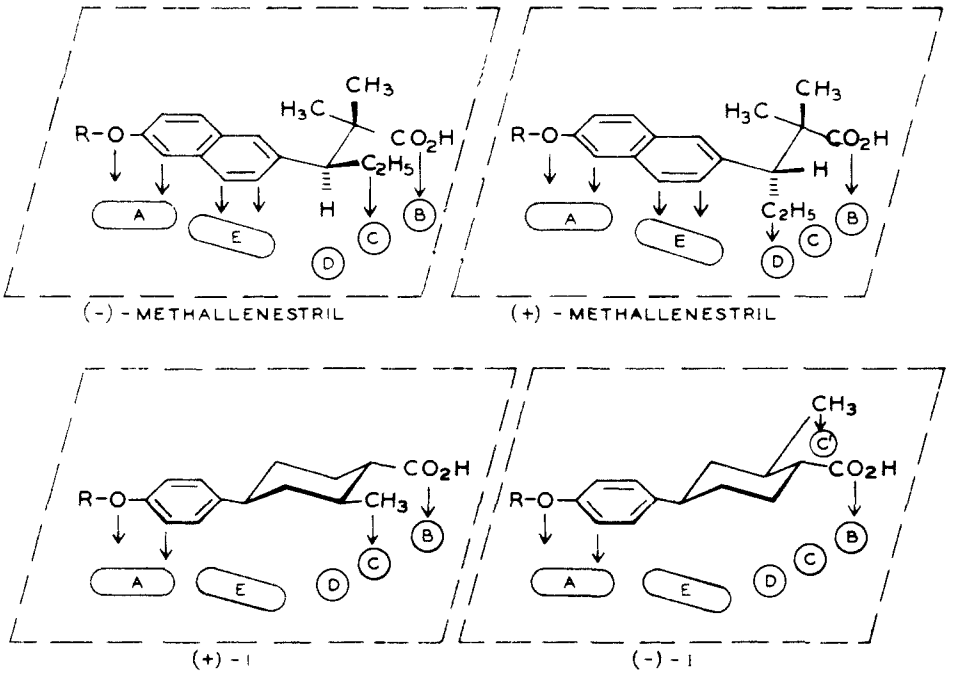


Figure 2. Model of Figure 1 showing superimposition of (+)- and (−)-methallenestril and of (+)- and (−)-1. As pointed out in the text, actual forms are probably those in which R = H; additionally, complexation with site B may involve a metabolized oxygenated form of the carboxyl moiety.

modate alkyl groups in the spatial relationship depicted in C′ in Figure 2, and in the accompanying paper we report a systematic placement of alkyl groups on the cyclohexane ring of (±)-1 which provides support for this hypothesis as well as giving insights into additional structural requirements at the receptor level for an estrogenic response.[12]

Experimental Section

Melting points are capillary and are uncorrected. Nmr spectra were obtained using a Varian Associates HA-100 spectrometer. All optical rotations were measured at 22° at a concentration of 10 mg/cm³ in $CHCl_3$ solutions using a Perkin-Elmer Model 141 polarimeter. Yields reported in ref 2 for the corresponding racemic compounds were generally duplicated.

Optical Purity Assay of (+)-*cis*-2-Methyl-4-ketocyclohexanecarboxylic Acid [(+)-2]. A sample of (+)-2† (80.5 mg, 0.52 mmol, $[\alpha]_D$ +12.8°) in benzene (2 ml) containing oxalyl chloride (0.25 ml) was stirred at 25° for 1.25 hr; the solution then was evaporated. To the residual oil was added methyl (−)-mandelate (87.9 mg, 0.53 mmol) in benzene (2 ml) and the resultant solution was refluxed for 18 hr. The solution was evaporated and the product was examined by nmr: nmr ($CDCl_3$) δ 5.94 [s, 1, $CO_2CH(Ph)CO_2CH_3$] and 3.70 ppm [s, 3, $CO_2CH(Ph)CO_2CH_3$]. Small peaks were also seen at δ 5.14 and 3.72 ppm for the corresponding peaks in unchanged methyl (−)-mandelate. To show that the assay is valid for determining optical purity in (+)- or (−)-2, the reaction was repeated using (±)-2 and methyl (−)-mandelate: nmr ($CDCl_3$) δ 5.92 and 5.94 [two singlets, 1, $CO_2CH(Ph)CO_2CH_3$ diastereomers] and 3.68 and 3.70 [two singlets, 3, $CO_2CH(Ph)CO_2CH_3$ diastereomers]; the two peaks in each pair were of equal intensity.

***cis*-4-(*p*-Methoxyphenyl)-2-methyl-Δ³(Δ⁴)-cyclohexenecarboxylic acid [(+) isomers 3a and 3b]** was prepared from the keto acid (−)-2 and *p*-methoxyphenylmagnesium bromide as described previously for the racemic compounds.[2] The crude ene acid was recrystallized twice (MeCN) to give **3a**: mp 147–150.5°; indicated by glpc[2] to contain a ratio of Δ^4/Δ^3 isomers of approximately 2.5 (estimate from peak heights); $[\alpha]_D$ +6.8°. Evaporation of the filtrate from **3a** gave **3b**: mp 138–153°; similarly indicated as a mixture of Δ^4/Δ^3 in an approximate ratio of 1.4; $[\alpha]_D$ +47.8°. **(−) isomer 3c** was similarly prepared from (+)-2 and the Grignard reagent. The crude ene acid was recrystallized twice (MeCN) to yield **3c**: mp 146.5–150°; Δ^4/Δ^3 ratio, approximately 2.7; $[\alpha]_D$ −4.0°.

(+)-4β-(*p*-Methoxyphenyl)-2β-methylcyclohexane-α-carboxylic Acid [(+)-1]. The acids **3a** and **3b** were hydrogenated separately by the procedure described for the racemic acid[2] to yield, after evaporation of the solvent, samples of 4 having $[\alpha]_D$ of −22.7 and −23.0°, respectively; these samples contained about 4% each of the C-4 epimer.[2] The combined samples were converted to the methyl ester which was epimerized and then hydrolyzed to the title compound as described previously;[2] the crude product was recrystallized twice (MeCN) to give (+)-1 of constant rotation: $[\alpha]_D$ +17.5°; mp 122–125° (turbid melt, clears at 153°).‡ As indicated in the text, the assignment of absolute configuration is to be considered provisional.

(−)-4α-(*p*-Methoxyphenyl)-2α-methylcyclohexane-β-carboxylic Acid [(−)-1]. A. From Synthesis. The acid **3c** was hydrogenated as above to yield, after evaporation of the solvent, 4 having $[\alpha]_D$ +22.7°. This product was treated as above to yield (−)-1: $[\alpha]_D$ −17.5°; mp 123–127° (turbid melt, clears at 153°).‡

B. From Resolution. A solution of oxalyl chloride (25 ml) and (±)-1 (12.0 g, 0.048 mol) in benzene (250 ml) was stirred at 20° for 2.5 hr. The solvent was evaporated and to the residual acid chloride was added toluene (180 ml) and testosterone (13.8 g, 0.048 mol). The mixture was heated at reflux for 17 hr and then was worked up to yield an oil; chromatography on silica gel (elution with 95:5 toluene–Et_2O) yielded 22.3 g (90%) of diastereoisomeric testosteronyl esters of (±)-1. Successive recrystallizations from MeCN, EtOAc, MeCN (twice), and EtOH gave 3.02 g of white needles: mp 150–151°; $[\alpha]_D$ +85°; melting point and rotation unchanged with subsequent recrystallization.

A mixture of this ester (2.80 g, 0.005 mol) in EtOH (40 ml) and H_2O (5 ml) containing KOH (0.50 g, 0.009 mol) was stirred at reflux for 20 hr. Usual work-up gave (−)-1 (0.49 g, 38%); recrystallized (MeCN) to $[\alpha]_D$ −13.2°. The unhydrolyzed ester was recovered and treated similarly using 1.6 g of KOH in refluxing aqueous EtOH for 16 hr. The acid obtained (0.67 g, 51%) was recrystallized twice (MeCN) to yield (−)-1: $[\alpha]_D$ −17.7°; mp 122–123.5° (turbid melt as above, clearing at 153°).

Optical Purity Assay of (−)-1. The acid (−)-1 (25.3 mg, 0.10 mmol) from part B above was converted with oxalyl chloride to the acid chloride which then was heated with methyl (−)-mandelate (33.7 mg, 0.20 mmol) at 160° for 2.5 hr. The product was dissolved in toluene and the solution was filtered through alumina. The solvent was evaporated and the residue was examined by nmr: nmr ($CDCl_3$) δ 5.96 ppm [s, 1, $CO_2CH(Ph)CO_2CH_3$]. The racemic acid (±)-1 and methyl (−)-mandelate were treated in an identical procedure and the resultant product was analyzed: nmr ($CDCl_3$) δ 5.96 and 5.99 ppm [s, ½, s, ½, $CO_2CH(Ph)CO_2CH_3$ diastereomers].

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‡The racemic acid (±)-1 shows a similar melting pattern, melting sharply at 138–139.5° to a cloudy melt which clears at 153°. This behavior is not seen in the three racemic diastereoisomers related to (±)-1. The melting pattern seen with (±)-1 has been attributed to liquid crystal formation (personal communication, Dr. M. E. Neubert, Liquid Crystal Institute, Kent State University).